

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/47, A61K 38/17		A2	(11) International Publication Number: WO 96/40768																																																																																																						
			(43) International Publication Date: 19 December 1996 (19.12.96)																																																																																																						
(21) International Application Number: PCT/US96/09374 (22) International Filing Date: 7 June 1996 (07.06.96) (30) Priority Data: 08/481,888 7 June 1995 (07.06.95) US (60) Parent Application or Grant (63) Related by Continuation US Not furnished (CIP) Filed on Not furnished (71) Applicants (for all designated States except US): PERIODON- TIX, INC. [US/US]; 313 Pleasant Street, Watertown, MA 02171 (US). TRUSTEES OF BOSTON UNIVERSITY [US/US]; 147 Bay State Road, Boston, MA 02115 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): OPPENHEIM, Frank, G. [US/US]; 10 Heath Wood Lane, Chestnut Hill, MA 02167 (US). XU, Tao [CN/US]; 160 Pine Street, No. 9, Newton, MA 02166 (US). ROBERTS, F., Donald [US/US]; 2 Bridle Path Circle, Dover, MA 02030 (US). SPACCIAPOLI, Peter [US/US]; 9 High Road, Newbury, MA 01951 (US).		FRIDEN, Phillip, M. [US/US]; 32 Washington Street, Bedford, MA 01730 (US). (74) Agents: BROOK, David, E. et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US). (81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published Without international search report and to be republished upon receipt of that report.																																																																																																							
(54) Title: ANTI-FUNGAL AND ANTI-BACTERIAL HISTATIN-BASED PEPTIDES																																																																																																									
<table border="0"> <tr> <td>Histatin 1:</td> <td>1</td> <td>5</td> <td>10</td> <td>15</td> <td></td> </tr> <tr> <td></td> <td colspan="5">Asp-Pse-His-Glu-Lys-Arg-His-His-Gly-Tyr-Arg-Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 2:</td> <td></td> <td></td> <td></td> <td></td> <td>Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 3:</td> <td>1</td> <td>5</td> <td>10</td> <td>15</td> <td></td> </tr> <tr> <td></td> <td colspan="5">Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 4:</td> <td></td> <td></td> <td></td> <td></td> <td>Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 5:</td> <td colspan="5">Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 6:</td> <td colspan="5">Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 7:</td> <td></td> <td></td> <td></td> <td></td> <td>Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 8:</td> <td></td> <td></td> <td></td> <td></td> <td>Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 9:</td> <td></td> <td></td> <td></td> <td></td> <td>Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 10:</td> <td></td> <td></td> <td></td> <td></td> <td>Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Histatin 11:</td> <td></td> <td></td> <td></td> <td></td> <td>Lys-Arg-His-His-Gly-Tyr-Lys-Arg</td> </tr> <tr> <td>Histatin 12:</td> <td></td> <td></td> <td></td> <td></td> <td>Lys-Arg-His-His-Gly-Tyr-Lys</td> </tr> <tr> <td>Peptide 101:</td> <td></td> <td></td> <td></td> <td></td> <td>Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Peptide 102:</td> <td></td> <td></td> <td></td> <td></td> <td>Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> <tr> <td>Peptide 103:</td> <td></td> <td></td> <td></td> <td></td> <td>Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td> </tr> </table>				Histatin 1:	1	5	10	15			Asp-Pse-His-Glu-Lys-Arg-His-His-Gly-Tyr-Arg-Arg-Lys-Phe-His-Glu-Lys-His-His-					Histatin 2:					Arg-Lys-Phe-His-Glu-Lys-His-His-	Histatin 3:	1	5	10	15			Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-					Histatin 4:					Arg-Lys-Phe-His-Glu-Lys-His-His-	Histatin 5:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-					Histatin 6:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-					Histatin 7:					Arg-Lys-Phe-His-Glu-Lys-His-His-	Histatin 8:					Lys-Phe-His-Glu-Lys-His-His-	Histatin 9:					Arg-Lys-Phe-His-Glu-Lys-His-His-	Histatin 10:					Lys-Phe-His-Glu-Lys-His-His-	Histatin 11:					Lys-Arg-His-His-Gly-Tyr-Lys-Arg	Histatin 12:					Lys-Arg-His-His-Gly-Tyr-Lys	Peptide 101:					Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-	Peptide 102:					Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-	Peptide 103:					Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-
Histatin 1:	1	5	10	15																																																																																																					
	Asp-Pse-His-Glu-Lys-Arg-His-His-Gly-Tyr-Arg-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																								
Histatin 2:					Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																				
Histatin 3:	1	5	10	15																																																																																																					
	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																								
Histatin 4:					Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																				
Histatin 5:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																								
Histatin 6:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																								
Histatin 7:					Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																				
Histatin 8:					Lys-Phe-His-Glu-Lys-His-His-																																																																																																				
Histatin 9:					Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																				
Histatin 10:					Lys-Phe-His-Glu-Lys-His-His-																																																																																																				
Histatin 11:					Lys-Arg-His-His-Gly-Tyr-Lys-Arg																																																																																																				
Histatin 12:					Lys-Arg-His-His-Gly-Tyr-Lys																																																																																																				
Peptide 101:					Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																				
Peptide 102:					Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																				
Peptide 103:					Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																																																				
(57) Abstract																																																																																																									
Histatin-based peptides representing defined portions of the amino acid sequences of naturally occurring human histatins and methods for treatment of fungal or bacterial infection are described. These histatin-based peptides represent the active anti-fungal and anti-bacterial region of naturally occurring human histatins.																																																																																																									

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

-1-

ANTI-FUNGAL AND ANTI-BACTERIAL HISTATIN-BASED PEPTIDESGOVERNMENT SUPPORT

The invention described herein was supported in whole or in part by Grant No. DE07652 from the National
5 Institutes of Health, which have certain rights in the invention.

RELATED APPLICATIONS

This Application is a Continuation-in-Part of and claims priority to U.S. Serial No. 08/481,888, filed
10 June 7, 1995, which is a Continuation-in-Part of U.S. Serial No. 08/287,717, filed August 9, 1994, which is a File Wrapper Continuation of U.S. Serial No. 08/145,030, filed October 28, 1993 (now abandoned), which is a File Wrapper Continuation of U.S. Serial No. 07/786,571, filed
15 November 1, 1991 (now abandoned), the contents of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

The family of naturally occurring human histatins is a group of twelve low molecular weight, abundant in
20 histidine, peptides found in human submandibular and parotid salivary secretions (Oppenheim et al. (1986), J. Biol. Chem. 261: 1177-1182; Oppenheim et al. (1988), J. Biol. Chem. 263: 7472-7477; Troxler et al. (1990), J. Dent. Res. 69: 2-6). The primary structure of the major
25 family members (histatins 1, 3, and 5; 70-80% of the whole family) has shown that these proteins consist of 38, 32 and 24 amino acid residues, respectively. There is a high degree of homology among these three major histatins. Histatin 5 results from post-translational cleavage of
30 histatin 3. Many of the smaller members of the histatin

-2-

family may also, in fact, originate by post-translational proteolysis of histatins 1, 3 and 5 (Oppenheim et al. (1989), Human Saliva: Clinical Chemistry and Microbiology Vol. 1 CRC Press, Boca Raton, FL, ed. Tenovuo, J.O.; Lal et al. (1992), Arch. Oral Biol. 37: 7-13). The genes that encode histatins 1 and 3 have been localized chromosomally (vanderSpek et al., (1989), Am. J. Hum. Genet. 45: 381-387) and sequenced (Sabatini, L.M. et al. (1989), Biochem. Biophys. Res. Comm. 160:495-502). Histatins 1 and 3 appear to be derived from separate genes.

The three major human histatins exhibit specific antimicrobial activities towards diverse oral microbiota. These histatins, at physiological concentrations, are capable of killing Candida albicans in both blastopore and mycelial forms (Pollock, J.J. et al. (1984), Infect. Immun. 44:702-707; Xu, T. et al. (1991), Infect. Immun. 59 (8): 2549-2554). Histatins are also capable of killing oral bacteria, including Streptococcus mutans (MacKay, B.J. et al. (1984), Infect. Immun. 44:695-701; Xu, T. et al. (1990), J. Dent. Res. 69: 239), Porphyromonas gingivalis (Colon et al. (1993), J. Dent. Res. 72: 322) and Actinomyces viscosus (Kalpidis et al. (1992) J. Dent. Res. 72: 305).

Infection with the yeast Candida albicans is a prevalent and, in some cases, life-threatening condition affecting otherwise healthy and immuno-compromised patients. Candidal vaginitis is estimated to affect 15 to 55% of healthy young women. Candidal infections often occur in diabetics, during pregnancy, and following medication with antibiotics, steroid hormones, or oral contraceptives. (Tapper-Jones, L.M. et al. (1981) J. Clin. Pathol. 34:706-11; Sobel, J.D. et al. (1984) Infect. Immun. 44:576-580). Oral candidiasis is an early opportunistic infection of Acquired Immune Deficiency Syndrome (AIDS) in individuals infected with human immunodeficiency virus type

-3-

1, as well as a complication of radiation and chemotherapy in cancer patients. (Yeh, C.-K. et al. (1988) J. of Acquired Immune Deficiency Syndromes 1:361-366). In addition, candidal infection of denture wearers plays a primary role in dental stomatitis, a prevalent oral problem among the elderly. (Pollock, J.J. et al. (1990) NYS Dental J. 56:36-38). Candidal infections of skin and urethra are widespread problems. In patients in intensive care and immuno-compromised patients, systemic fungal infection often leads to death, since there are few safe and effective anti-fungal pharmaceuticals for intravenous use. (Burnie, J.P. et al. (1985) British Medical Journal 290:746-748). Similarly, infections with various bacterial species can cause severe disease states and even death.

Although several anti-fungal agents (e.g., clotrimazole, miconazole, ketoconazole, and nystatin) and anti-bacterial agents (penicillin, streptomycin, tetracycline and chlorhexidine) are currently available, these agents are not completely effective, can lead to drug resistant organisms and can produce adverse side effects. Many are not appropriate for oral or systemic administration. Thus, a potent, naturally occurring anti-fungal or anti-bacterial substance would provide a significant improvement in the treatment of microbial infection.

SUMMARY OF THE INVENTION

This invention is based on substantially pure peptides which have anti-candidal or anti-bacterial activity which are equivalent to that of naturally occurring histatins but are smaller in size. These peptides represent defined portions of the amino acid sequences of naturally occurring human histidine-rich salivary proteins called histatins, which will be referred to herein as histatin-based peptides. The histatin-based peptides of this invention

-4-

also include defined portions of the amino acid sequences of histatins with specific amino acid substitutions at specified positions of the sequences. As demonstrated herein, these histatin-based peptides have been shown to be superior, particularly on a weight basis, in anti-candidal or anti-bacterial activity over the naturally occurring histatins. Thus, this invention provides compositions for treatment of fungal or bacterial infection comprising histatin-based peptides with defined amino acid sequences. These peptides are derived from a specific histatin-based peptide having a specified 12 amino acid sequence. This peptide is designated as peptide 113 (SEQ ID NO: 18). Other peptides with significant anti-fungal or anti-bacterial activities have sequence portions of at least 8 amino acids from this histatin-based peptide or are homologs of peptide 113 with amino acid substitutions at particular positions in the peptide.

BRIEF DESCRIPTION OF FIGURES

Figure 1A-1D shows the amino acid sequences of human histatins and peptides 101, 102, 103, 104, 105, 113, 113-F4, 113-F5, 113-F12, 113-F4.5, 113-F4.5.12, 113-K6, 113-H8, 113-K6H8, 113-F8, 113-L4.5.12, 113-Y4.5.12, 113-Q2.10, 113-Q3.9, 113-Q2.3.9.10, 117, 118, 119, 120 and 129.

Figure 2 is a graph that shows the % killing of *C. albicans* blastoconidia as a function of the concentration of histatin-5, peptide 103, peptide 113 and peptide 129.

Figure 3 is a graph that shows the % killing of *C. Albicans* blastoconidia as a function of concentration of peptide 113, peptide 113-F4, peptide 113-F5, peptide 113-F12, peptide 113-F4.5 and peptide 113-F4.5.12.

Figure 4 is a graph that shows the % killing of *C. albicans* blastoconidia as a function of concentration of peptide 113, peptide 113-K6, peptide 113-H8, peptide

-5-

113-K6H8, peptide 113-F8, peptide 113 with an acetyl group on the N-terminus (NA) and peptide 113 with a carbamyl group on the N-terminus (NC).

Figure 5 is a graph that shows the % killing of
5 *C. albicans* blastoconidia as a function of concentration of peptide 113, peptide 113-L4.5.12, peptide 113-Y4.5.12, peptide 113-Q2.10, peptide 113-Q3.9 and peptide 113-Q2.3.9.10.

Figure 6 is a graph that shows the % killing of
10 *C. albicans* blastoconidia as a function of concentration of peptide 113 and peptide 113-F4.5.12 at pH 7.4 and at pH 4.0.

Figure 7 is a graph that shows the amount of growth inhibition of *P. gingivalis* as a function of time for
15 histatin 5, peptide 103, peptide 113 and peptide 129, as well as when no histatin-based peptide is present.

Figure 8 is a graph that shows the % killing of
P. aeruginosa as a function of concentration of peptide 113, peptide 113-K6, peptide 113-H8, peptide
20 113-K6H8, peptide 113-F4.5.12 and peptide 113 with an acetyl group on the N-terminus (NA).

Figure 9 is a graph that shows the % killing of
P. aeruginosa as a function of concentration of peptide 113, peptide 113-Y4.5.12, peptide 113-L4.5.12, peptide
25 113-Q2.10, peptide 113-Q3.9 and peptide 113-Q2.3.9.10.

Figure 10 is a graph that shows the % inhibition of clostripain activity as a function of the concentration of histatin 5, peptide 101, peptide 103, peptide 105, peptide 118, peptide 119, peptide 120 and peptide 129.

30 Figure 11 is a graph that shows the % inhibition of clostripain activity as a function of the concentration of peptide 113 and peptide 113-F4.5.12.

-6-

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to peptides which have anti-fungal or anti-bacterial activity, in which the amino acid sequences represent defined portions of the amino acid sequences of naturally occurring human histidine-rich salivary proteins called histatins. (Histatins are also referred to in the literature as histidine-rich proteins or HRP's.) Histatins are major salivary proteins which are synthesized in the parotid and submandibular-sublingual secretory glands of humans and Old World monkeys. (Azen, E.A. (1978) Biochem. Genet. 16:79-99). Histatins are believed to be part of an extraimmunologic defense system of the oral cavity. The anti-fungal activity of histatins, as well as their inhibitory effect on several oral bacteria (such as the cariogenic *Streptococcus mutans* and the periodontal pathogen *Porphyromonas gingivalis*), have been demonstrated *in vitro*. In addition, the observation that polyhistidine peptides inactivate herpes simplex virus *in vitro* and that whole saliva contains inhibitors of human immunodeficiency virus suggests the possibility that histatins may have anti-viral activity. These *in vitro* studies support potential clinical use of compositions containing histatins or histatin-based peptides for the treatment of local and systemic candidal infection, oral bacterial diseases, such as caries and periodontitis, systemic bacterial infection and viral infection. Vaginal, urethral, mucosal, respiratory, skin, ear, oral or ophthalmic fungal or bacterial infections are particularly susceptible to histatin-based peptide therapy. Microbes which are specifically amenable to histatin-based peptide therapy are:

- a) *Candida albicans*;
- b) *Actinomyces actinomycetemcomitans*;
- c) *Actinomyces viscosus*;
- d) *Bacteroides forsythus*;

-7-

- e) *Bacteriodes fragilis*;
- f) *Bacteriodes gracilis*;
- g) *Bacteriodes ureolyticus*;
- h) *Campylobacter concisus*;
- 5 i) *Campylobacter rectus*;
- j) *Campylobacter showae*;
- k) *Campylobacter sputorum*;
- l) *Capnocytophaga gingivalis*;
- m) *Capnocytophaga ochracea*;
- 10 n) *Capnocytophaga sputigena*;
- o) *Clostridium histolyticum*;
- p) *Eikenella corrodens*;
- q) *Eubacterium nodatum*;
- r) *Fusobacterium nucleatum*;
- 15 s) *Fusobacterium periodonticum*;
- t) *Peptostreptococcus micros*;
- u) *Porphyromonas endodontalis*;
- v) *Porphyromonas gingivalis*;
- w) *Prevotella intermedia*;
- 20 x) *Prevotella nigrescens*;
- y) *Propionibacterium acnes*;
- z) *Pseudomonas aeruginosa*;
- aa) *Selenomonas noxia*;
- bb) *Staphylococcus aureus*;
- 25 cc) *Streptococcus constellatus*;
- dd) *Streptococcus gordonii*;
- ee) *Streptococcus intermedius*;
- ff) *Streptococcus mutans*;
- gg) *Streptococcus oralis*;
- 30 hh) *Streptococcus pneumonia*;
- ii) *Streptococcus sanguis*;
- kk) *Treponema denticola*;
- ll) *Treponema pectinovorum*;
- mm) *Treponema socranskii*;
- 35 nn) *Veillonella parvula*; and

-8-

oo) *Wolinella succinogenes*.

The human histatin proteins have been isolated and sequenced. They have been shown to be a family of twelve related low molecular weight proteins. Comparison of the amino acid sequences of the histatins suggests that histatin 2 and histatins 4-12 may have originated from specific proteolytic cleavage of histatin 1 and histatin 3, respectively. (Oppenheim, F.G. et al. (1988), J. Biol. Chem. 263:7472-77; Troxler, R.F. et al. (1990), J. Dent. Res. 69(1):2-6). Cloning and sequence analysis of histatin cDNAs further suggest that the histatins are encoded by two homologous genetic loci, whose primary products are histatins 1 and 3. (Sabatini, L.M. et al. (1989), Biochem. Biophys. Res. Comm. 160:495-502; Vanderspek, J.C. et al. (1990), Arch. Oral Biol. 35(2):137-43).

The amino acid sequences of the anti-fungal and anti-bacterial peptides of this invention represent all or defined portions of the amino acid sequence of peptide 113 (SEQ ID NO: 18). In addition, the anti-fungal and anti-bacterial peptides of this invention include all or defined portions of peptide 113 (SEQ ID NO: 18) with amino acid substitutions at particular positions of the peptide.

Preferred embodiments of this invention are peptide 113 itself (SEQ ID NO: 18); fragments of peptide 113 containing at least an 8 amino acid sequence from this peptide; an amino acid sequence of at least 8 amino acids from peptide 113 where the glycine at position 6 is replaced by lysine, arginine or another basic amino acid; an amino acid sequence of at least 8 amino acids from peptide 113 where the lysine at position 8 is replaced by histidine, phenylalanine or another hydrophobic amino acid; an amino acid sequence of at least 8 amino acids from peptide 113 where one or more of the histidines at

-9-

positions 4, 5 and 12 is (are) replaced by phenylalanine, tyrosine, leucine or another hydrophobic amino acid; an amino acid sequence of at least 8 amino acids from peptide 113 where one or both of the lysines at positions 2 and 10 is (are) replaced by glutamine, arginine or a combination of glutamine and arginine (when both lysines are replaced); and an amino acid sequence of at least 8 amino acids from peptide 113 where one or both of the arginines at positions 3 and 9 is (are) replaced by glutamine, lysine or a combination of glutamine and lysine (when both arginines are replaced). Combinations of these amino acid replacements in an amino acid sequence of at least 8 amino acids from peptide 113 are all preferred embodiments of the invention provided that a combination of 4 glutamines or any other group of 4 non-basic amino acids at positions 2, 3, 9 and 10 does not occur.

Specific preferred embodiments of this invention are peptide 113 itself (SEQ ID NO: 18), histatin 11 (SEQ ID NO: 11), peptide 129 (SEQ ID NO: 23), peptide 117 (SEQ ID NO: 19), peptide 118 (SEQ ID NO: 20), peptide 119 (SEQ ID NO: 21), peptide 120 (SEQ ID NO: 22), peptide 113-F4 (SEQ ID NO: 24), peptide 113-F5 (SEQ ID NO: 25), peptide 113-F12 (SEQ ID NO: 26), peptide 113-F4.5 (SEQ ID NO: 27), peptide 113-F4.5.12 (SEQ ID NO: 28), peptide 113-K6 (SEQ ID NO: 29), peptide 113-H8 (SEQ ID NO: 30), peptide 113-K6H8 (SEQ ID NO: 31), peptide 113-F8 (SEQ ID NO: 32), peptide 113-L4.5.12 (SEQ ID NO: 33), peptide 113-Y4.5.12 (SEQ ID NO: 34), peptide 113-Q2.10 (SEQ ID NO: 35), and peptide 113-Q3.9 (SEQ ID NO: 36). The amino acid sequences of these preferred peptides are shown in Figure 1A-1D. Combinations of two or more of these peptides are also effective as anti-fungal or anti-bacterial compositions and are included as compositions of the invention. However, the combination of these peptides where glutamine occurs at

-10-

positions 2, 3, 9 and 10, i.e. peptide 113-Q2.3.9.10 (SEQ ID NO: 37) is not a specifically preferred embodiment.

The peptides can be obtained from a naturally occurring source of histatin or they can be chemically synthesized or obtained by recombinant DNA techniques as expression products from cellular sources. These peptides can be altered by minor chemical modifications, such as by adding small substituents or by modifying one or more of the covalent bonds within or between the amino acid residues, without significantly diminishing the anti-fungal or anti-bacterial activities of the peptides. Quite useful modifications are the addition of a substituent to either the amino terminus, the carboxyl terminus or to both ends of the peptide. These substituent addition modifications appear to stabilize the peptide in its active form and to aid in the prevention of enzymatic degradation of these peptides. These substituent groups are added to the amine, at the amino terminus, or to the carboxyl group, at the carboxyl terminus. The substituent groups can be somewhat bulky and may include one or more natural or modified amino acids. Particularly useful modifications are acetylation or carbamylation of the amino terminus of the peptide or amidation of the carboxyl terminus of the peptide. A combination of both modifications is especially useful. Such modifications appear to increase the biological half-life of the peptides before degradation, encapsulation, internalization or excretion occurs.

The peptides described herein were tested in assays designed to measure separately their effectiveness in killing of blastoconidia of *C. albicans* in inhibiting the growth of *P. gingivalis*, in inhibiting hemagglutination caused by *B. forsythus*, and in inhibiting clostripain activity. These assays are indicative of anti-fungal and anti-bacterial activities of the histatin-based peptides of the present invention. When tested in these assays, the

-11-

histatin-based peptides of this invention were found surprisingly to have superior anti-candidal and anti-bacterial activity, particularly on an equivalent weight basis, when compared with histatin 5 as well as with
5 histatin-based peptides 101-105. These anti-fungal and anti-bacterial activities are surprising in view of their size and truncated peptide form.

The following is a description of the histatin-based peptides, the antifungal activities of the histatin-based
10 peptides as measured in assays for killing of *Candida* blastoconidia, and the anti-bacterial activities of the histatin-based peptides as measured in assays for inhibition of *P. gingivalis* growth, inhibition of hemagglutination caused by *B. forsythus* and inhibition of
15 clostripain enzyme activity.

Histatin 5 and Histatin-Based Peptides

Histatin 5 and the histatin-based peptides 101, 102, 103, 104, 105, 111, 113, 113-F4, 113-F5, 113-F12, 113-F4.5, 113-F4.5.12, 113-K6, 113-H8, 113-K6H8, 113-F8, 113-L4.5.12,
20 113-Y4.5.12, 113-Q2.10, 113-Q3.9, 113-Q2.3.9.10, 117, 118, 119, 120 and 129 were chemically synthesized. The amino acid sequences of histatin 5 and the synthesized histatin-based peptides are shown in Figure 1A-1D.

Anti-Fungal Activities of Histatin-Based Peptides

25 *C. albicans* is a dimorphic yeast. It can exist in a yeast or blastoconidial form, which upon germination develops into the hyphal or germinated form. While the germinated form is considered to be more invasive, most of the *C. albicans* isolates harvested from the oral cavities
30 of healthy individuals appear to be in the blastoconidial form. (Arendorf, T.M. et al. (1980), Arch. Oral Biol. 25:1-10; Gow, N.A.R. et al. (1987), Crit. Rev. Microbiol. 15:73-78; Odds, F.C. (1988), Candida and Candidosis, 2nd

-12-

ed., Bailliere Tindall, London, England). Anti-fungal activity of synthetic histatin 5, histatin-based peptide 113, peptides based on portions of the amino acid sequences of histatin-based peptide 113 and peptides derived from

5 histatin-based peptide 113 with specified amino acid substitutions was measured in assays designed to test the effectiveness of the peptides against the blastoconidia form of *Candida* (Table 1). These assays, which measure killing of blastoconidia of *C. albicans*, are described in

10 Xu et al., which is herein incorporated by reference. (Xu, T. et al. (1991), Infect. Immun. 59(8):2549-2554). Peptide 113 was found to be about equipotent with histatin 5, demonstrating its anti-fungal activity despite its size in comparison with histatin 5. Peptides 101-105

15 demonstrated fungicidal activity comparable to that of histatin 5 and histatin-based peptide 113. Histatin 11, peptides 117-120 and peptide 129 all have demonstrable fungicidal activity even though they are smaller than peptide 113. These latter peptides appear to have the

20 amino acid sequences of peptide 113 (and histatin 5) which are required for anti-fungal activity. The anti-fungal potency of the histatin-based peptide appears to be a function of both the size and the amino acid sequence of the respective peptide. In particular, the anti-fungal

25 potency of human histatins appears to reside in peptide 113 with selected subpeptides of peptide 113 maintaining at least partial anti-fungal activity.

Modifications of peptide 113 by making particular types of amino acid substitutions in this peptide result in

30 peptides that retain anti-fungal activity and in many instances display enhanced anti-fungal activity in comparison to peptide 113. For example, replacements of histidine with phenylalanine at positions 4, 5 or 12, either singly or in combination, result in peptides with

35 increased anti-fungal activities in comparison to peptide.

-13-

113. Likewise, replacements of the glycine with lysine at position 6 or the lysine with histidine or phenylalanine at position 8, either singly or in combination, result in other peptides with noticeably increased fungicidal activities in comparison to peptide 113. Acetylation or carbamylation of the N-terminus of peptide 113 also yields modified peptide 113 peptides with significant anti-fungal activity.

An additional feature of the directed amino acid substitutions of peptide 113 is that particular types of amino acid substitutions result in peptides with enhanced activities, e.g. anti-fungal, at a pH other than neutral. For example, the substitution of histidine with phenylalanine at positions 4, 5 and 12 resulted in peptides with significant anti-fungal activity at pH 4.0 while peptide 113 is essentially devoid of anti-fungal activity at this lower pH. Similar modifications of the peptides of this invention, such as peptide 113, to allow the peptides to have anti-microbial activity at pHs other than neutral values are included in the present invention.

Peptide sequences containing permutations of the amino acid sequences of native histatins and modified peptides can be produced by known methods, such as recombinant DNA techniques and solid-phase synthesis. Cloned DNA encoding the human histatins may be obtained as described by Sabatini et al. or Vanderspek et al., whose teachings are incorporated herein by reference. (Sabatini. L.M. et al. (1989), Biochem. Biophys. Res. Comm. 160:495-502; Vanderspek, J.C. et al. (1990), Arch. Oral Biol. 35(2):137-43). cDNA encoding the histatin-based peptides can be cloned by recombinant DNA techniques, for instance, by using degenerate oligonucleotides based on the amino acid sequence of the histatin-based peptides as primers for polymerase chain reaction amplification. Alternatively, oligonucleotides encoding histatins or histatin-based

-14-

peptides can be synthesized chemically using commercially available equipment. They can then be made double-stranded and cloned into vectors for amplification in prokaryotic or eukaryotic host cells.

- 5 Histatin-based peptides can be produced in a variety of expression vector/host systems, which are available commercially or can be reproduced according to recombinant DNA and cell culture techniques. The vector/host expression systems can be prokaryotic or eucaryotic, and
10 can include bacterial, yeast, insect, mammalian, and viral expression systems. The construction of expression vectors encoding histatin-based peptides, transfer of the vectors into various host cells, and production of peptides from transformed host cells can be accomplished using genetic
15 engineering techniques, as described in manuals such as Molecular Cloning and Current Protocols in Molecular Biology, whose teachings are incorporated herein by reference. (Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989), Molecular Cloning, 2nd ed., Cold Spring Harbor
20 Laboratory Press; Ausubel, F.M. et al., eds., Current Protocols in Molecular Biology, New York; Greene Publishing Associates and Wiley-Interscience)

- Modified histatin based peptides, such as particular amino acid substitutions of peptide 113, can be synthesized
25 chemically, or be produced from cloned DNAs containing mutated nucleotide sequences. Histatin-based peptides encoded by expression vectors may be modified due to post-translational processing in a particular expression vector/host cell system. (See, e.g., Wold, F. (1981), Ann. Rev. Biochem. 50:783-814). Histatin-based peptides may
30 also be modified by chemical alteration of amino acid side-chain groups, or by other covalent modification. (See, e.g., Glazer, A.N. et al. (1975), Chemical Modification of Proteins, North Holland; Katre, N.V. et al. (1987), Proc. Natl. Acad. Sci. USA 84:1487)
35

-15-

Therapeutic Applications

The histatin-based peptides of this invention, representing defined portions of the amino acid sequence of histatin-based peptide 113: peptide 113 itself, histatin
5 11, peptide 117, peptide 118, peptide 119, peptide 120 and peptide 129, and modified peptide 113 such as peptide 113-F4, peptide 113-F5, peptide 113-F12, peptide 113-F4.5, peptide 113-F4.5.12, peptide 113-K6, peptide 113-H8, peptide 113-K6H8, peptide 113-F8, peptide 113-L4.5.12,
10 peptide 113-Y4.5.12, peptide 113-Q2.10, peptide 113-Q3.9, can be used in compositions and methods of treatment for fungal, and in particular, candidal infection, or for bacterial infection. These methods of treatment for fungal or bacterial infection apply to preventive treatment as
15 well. The compositions may contain combinations of histatin-based peptides, in order to obtain maximum activity against all developmental forms of the fungus. The ionic strength, presence of various mono- and divalent ions, and pH of the compositions may be adjusted to obtain
20 maximum anti-fungal or anti-bacterial activity of the histatin-based peptides, as described in Xu et al. (Xu, T. et al. (1991), Infect. Immun. 59(8):2549-54). Carriers appropriate for administration of anti-fungal agents to the vagina, the urethra, the ear, the oral cavity, the
25 respiratory system, the ophthalmic region, various mucosal regions and skin are known, and described, for instance, in US 4,725,576 (Fungicidal Polypeptide Compositions Containing L-His and Methods for Use Therefor by J.J. Pollock and B.J. MacKay, February 16, 1988). Compositions
30 for treatment of systemic infection can be administered by various routes, such as intravenously or subdermally.

Expression vectors encoding the above-mentioned peptides can be used in compositions and methods for anti-fungal or anti-bacterial treatment. Expression
35 vectors may be administered in compositions which introduce

-16-

genetic material encoding histatin-based peptides into cells of the patients. For example, recombinant expression vectors based on retroviruses or adenovirus vaccines may be used to infect patients.

5 A method of anti-fungal or anti-bacterial therapy using the above-described expression vectors is bacterial substitution therapy. Bacterial substitution therapy can be used to treat fungal or bacterial infection of areas in the urinary/reproductive, respiratory and/or gastro-
10 intestinal tracts of a patient. The therapy comprises the following: 1) transforming a particular bacterium with DNA comprising an expression vector which encodes a histatin-based peptide described above, thereby producing transformed cells; 2) selecting transformed cells which
15 express the peptide encoded by the expression vector, thereby obtaining transformed cells which express a histatin-based peptide; and 3) administering transformed cells which express a histatin-based peptide in an appropriate carrier to the infected area.

20 One application of bacterial substitution therapy is treatment of fungal or bacterial infections of the oral cavity. A number of species of the oral bacterial *Streptococcus* can be used as vehicles for the expression vectors. For example, recombinant *S. lactis* has been used
25 in oral immunization of mice against a heterologous antigen. (Iwaki, M. et al. (1990), Infect. Immun. 58(9):2929-34). Other oral bacteria which can be used as vehicles for the expression vectors, plasmids for constructing expression vectors capable of amplification in
30 oral bacterial host cells, transformation methods, and administration of compositions containing oral bacteria to humans have been described. (See, e.g., Kuramitsu, H.K. et al. (1984), J. General Microbiology 130:2497-2500; LeBlanc, D.J. et al. (1978), Proc. Natl. Acad. Sci. USA
35 75(7):3484-3487; Macrina, F.L. et al. (1980),

-17-

J. Bacteriology 143(3):1425-1435; Kuramitsu, H.K. et al. (1982), Infect. Immun. 36(1):435-436; Svanberg, M. et al. (1984), Infect. Immun. 43(3):817-821).

The compositions and methods for treatment of fungal or bacterial infections discussed above are not limited to use in humans, but can have veterinary applications as well.

Furthermore, the above-described compositions and methods for treatment of fungal infection can also be used for treatment of bacterial infections (e.g., of *S. mutans*, *P. aeruginosa* or *P. gingivalis*) and viral infections (e.g., of herpes simplex virus or human immunodeficiency virus type 1).

Hemagglutination Activity of Bacteria and Histatin

Inhibition of this Activity

Even though the association between hemagglutination activity and adherence on host cells in the oral environment is not clear, it is generally accepted that hemagglutination activity is an indicator for colonizing ability of bacteria. Periodontal pathogens must adhere to other bacteria and host cells in order to express their noxious destructive potential upon periodontal tissues. Hemagglutinin is thought to be involved in bacterial colonization. Proteases such as collagenase, sialidase, and trypsin-like protease are involved in the degradation of host tissues, and low-molecular weight toxic products such as butyric and propionic acids which are cytotoxic are produced. In a similar fashion, *B. forsythus* has been shown to possess sialidase and trypsin-like protease. Sialidase has the ability of altering the host response to periodontal microorganisms. For instance, it cleaves sialic acid from erythrocytes and leukocytes which results in removal of these cells from the circulation. It also decreases the ability of IgG to bind complement, decreases

-18-

collagen production, and stimulates lymphocytes. Trypsin-like protease can cleave a variety of synthetic substrates. It is so called because it can hydrolyse the synthetic substrate benzoyl-DL-arginine-naphthylamide (BANA), used for
5 detection of trypsin activity. *P. gingivalis*, *B. forsythus* and *T. denticola* possess strong BANA hydrolase activity. *B. forsythus* also possesses hemagglutinin(s).

Ability for adherence on erythrocytes is of great importance in the interactions of periodontal pathogens
10 with the host. The close proximity of these bacteria with the host tissues as well as with erythrocytes that bathe the periodontal pocket during progression of the disease, indicates multiple interrelations between these elements. Additionally, periodontal microbes require heme-containing
15 products for their survival and multiplication and this need dictates interactions with cells such as erythrocytes that are rich in these compounds. *P. gingivalis* has been shown to possess both hemagglutinin(s) and hemolysin that provide attachment on erythrocytes and utilization of heme-
20 compounds.

It has been shown (Murakami et al. (1990) Arch. Oral Biol. 9: 775) that histatin 5 and histatin 8 inhibit hemagglutination of *P. gingivalis* 381. Complete hemagglutination inhibition was reported for histatin 5 at
25 a concentration of 5 nmole/ml. Thus, it appears that histatins and, more importantly, histatin-based peptides can play a role in inhibiting bacterial growth and deleterious activity in the periodontal region.

Clostripain Inhibition by Histatin-Based Peptides

30 Clostripain is an endopeptidase enzyme synthesized by *Clostridium histolyticum*. This enzyme, with its protein degradative activity, can be inhibited by histatin 5 and by histatin-based peptides (see Table 1). Thus, histatin-based peptides can inhibit bacterial function by inhibiting

-19-

bacterial enzymes which are essential for the bacterial viability.

EXAMPLE 1 MATERIALS AND METHODS

A. Isolation and Chemical Synthesis of Histatin-Based Peptides

Isolation and amino acid sequence determination of human histatins were performed as described in Oppenheim et al., whose teachings are herein incorporated by reference. (Oppenheim, F.G. et al. (1988), J. Biol. Chem. 263(16):7472-7477). Human parotid secretion from healthy adults was stimulated using sour lemon candies, collected with Curby cups in ice-chilled graduated cylinders, pooled, dialyzed and lyophilized. Total protein in human parotid secretion was subjected to fractionation on Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, CA) developed in 0.05 M ammonium formate buffer, pH 4.0. The protein fraction enriched with histatins was further purified using reversed-phase high-performance liquid chromatography on a C₁₈ column. Purified histatins were evaporated to dryness, dissolved in deionized water, quantified by amino acid analysis, lyophilized, and stored at -20°C until use.

Histatin-based peptides were synthesized by the solid phase method of Merrifield. (Merrifield, B. (1986) Science 232:341-47). Peptides were synthesized by a MilliGen/Bioresearch Sam-Two Peptide Synthesizer using Fmoc L-amino acid kits (Millipore, Bedford, MA) and purified on a TSK ODS-i20T C₁₈ column (5 µm, 4.6 X 250 mm) using RP-HPLC (Pharmacia-LKB). The purified peptides were quantified by amino acid analysis on a Beckman System 6300 amino acid analyzer.

B. C. albicans Killing

(1) C. albicans Stock

-20-

A well-described strain of *C. albicans* was used in the bioassay. This strain, ATCC 44505, was originally isolated from the human oral cavity. Cultures were stored at 4°C on Sabouraud dextrose agar plates (Difco Laboratories, Detroit, MI) until use. Stationary phase growth cells were obtained following growth at 30°C for 18 h on Sabouraud dextrose agar plates. Colonies were harvested and suspended in 10 mM potassium phosphate buffer (PPB), pH 7.4.

To initiate log phase growth, an aliquot of stock *C. albicans* was suspended in Sabouraud dextrose broth (Difco) and incubated at 30°C in a shaking water bath. The growth phase was determined by taking aliquots of the culture at one hour intervals to monitor the optical density (O.D.) at 560 nm. Early log phase was obtained at 4 to 6 h, indicated by an O.D. of about 0.6. Log phase cells were harvested and utilized in the blastoconidia killing assay in a manner identical to that described for stationary phase cells. A final concentration of 10^5 cells/ml (either stationary or log phase fungus) was used in all assays.

(2) Suspension Buffers

The standard suspension buffer utilized in the blastospore killing assay was 0.01 M PPB, pH 7.4. An alternate suspension buffer, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acids (HEPES; Sigma Chemical Co., St. Louis, MO), pH 7.4, can also be utilized.

(3) Bioassays

The following assay was used to evaluate the effects of histatins on the killing of blastoconidia of *C. albicans*.

- a. For the killing of blastoconidia assay, 50 μ l aliquots of cells (2×10^5 cells/ml) diluted in suspension buffer were allowed to attach to a

-21-

polystyrene 96-well micro-titer plate (COSTAR, Cambridge, MA) for 15 min at room temperature, and then incubated with an equal volume of a histatin or histatin peptide in suspension buffer for 1 h at 37°C. Controls were carried out in the absence of the histatin or histatin peptide. After incubation, wells were washed three times by centrifugation at 1,000xg for 5 min and covered with aliquots of molten Sabouraud dextrose broth (Difco) containing 2% agarose (Sigma) at 45°C. The plate was then incubated at 30°C for 8 h. Under such conditions, live cells will divide and begin to form colonies, while dead cells will remain as single cells. To determine the percentage of blastoconidia killed, a total of 100 single cells and/or colonies were counted under a Nikon inverted microscope at 400x magnification and the extent of killing was calculated using the formula: $[1 - (\text{number of colonies in treated sample}) / (\text{number of colonies in control})] \times 100\%$.

(4) Statistical Analysis

Data were obtained by calculating the mean and standard deviation from triplicate assays. From the dose response relationship, doses effecting a 50% killing (LD₅₀) were determined.

C. BACTERIAL GROWTH INHIBITION AND CELL KILLING ASSAYS

(1) Bacterial Strains and Culture Conditions

(a) The bacteria used in one investigation, *Porphyromonas gingivalis* strain A7A1-28, is a typical key pathogenic organism associated with destructive periodontal diseases. The bacteria were multiply

-22-

subcultured in Enriched Todd Hewitt broth (ETHB, Difco Lab., Detroit, MI). Microorganisms were stored in the same broths containing 20% and 50% glycerol, at -20°C and -70°C, respectively. These served as stock cultures from which all preparations originated.

Working stock cultures were maintained by weekly transfer to Brain Heart Infusion Anaerobic Sheep Blood Agar plates (BHIA, Becton Dickinson and Co., Cockeysville, MD), and Trypticase Soy Anaerobic Sheep Blood Agar plates (TSA, Becton Dickinson and Co., Cockeysville, MD). Plates were incubated for 3 to 4 days under strictly anaerobic conditions. For the bacteriostatic assay, bacteria were collected from plates, inoculated into the aforementioned broth and grown at 37°C, under strictly anaerobic conditions for 24 to 48 hours.

(b) Two other bacterial species were used in a bacterial cell killing assay system. These bacterial species were *Streptococcus mutans* strain SJ32 and *Pseudomonas aeruginosa* ATCC Accession Number 27853. The assays were performed using liquid overnight cultures (nutrient broth for *P. aeruginosa*; Todd Hewitt broth for *S. mutans*) growth media from frozen stocks of these bacterial species. In the assay, the bacteria were diluted into assay buffer (10 mM Potassium Phosphate, pH 6.0 with 20 mM NaCl for *P. Aeruginosa*; and 10 mM Potassium Phosphate, pH 5.2 with 20 mM NaCl for *S. mutans*) to a concentration of 2×10^5 cfu/ml (1×10^9 cfu/OD/ml) and combined with an equal volume (250 μ l) of peptide to produce 500 μ l incubation mixture with a final concentration of 10^5 cfu/ml. Controls constituted buffer and bacteria but no peptide. After incubation at 37°C (30 minutes incubation for *P. aeruginosa*; and 60 minutes incubation for *S. mutans*), the mixtures were plated

-23-

onto agar media (nutrient agar for *P. aeruginosa*; and Todd Hewitt media with 0.5% glucose for *S. mutans*) and incubated at 37°C until colonies developed. The mean number of colonies was determined from a minimum of 4 plates and percent killing was determined by comparing the colony number arising from control cultures versus the colony number arising from peptide-containing assay mixtures.

(2) Microdilution Bacteriostatic Assay

A modification of the typical microdilution assay (Rotilie et al., 1975) for the determination of minimal inhibitory concentration (MIC) of antimicrobial agents was utilized to investigate the bacteriostatic activity of the peptides. A standardized bacterial inoculum (*P. gingivalis*) was exposed to serially diluted antimicrobial peptides in an enriched broth medium that was suitable for the growth of anaerobic bacteria. The test was adapted for use in the 96-well microtiter plates. Results with the microdilution method have been shown to be comparable to the other known techniques for antimicrobial susceptibility such as the dilution method, the agar dilution method, and the broth-disk elution method (Rosenblatt et al., 1979). In the typical assay, the microtiter plate was observed at multiple time points after incubation for visible growth. The modification introduced here was based on the spectrophotometric reading of the microtiter plate after incubation.

Microorganisms from cultures maintained in the aforementioned plates were inoculated into 5 ml of the above-mentioned broths and cultured overnight at 37°C under strictly anaerobic conditions with continuous agitation on a minishaker (IKA-Labortechnik, Staufen

-24-

i. Br., Germany). The bacteria were grown until reaching the late log phase and were then suspended in the same broths to an optical density (O.D.) of 0.1 at 560 nm. The peptides were diluted in 0.01 M phosphate buffered saline (PBS), pH 7. Forty μ l aliquots of peptide dilutions were added in each well of a U-bottom microtiter plate (Costar, Cambridge, MA) to give final concentrations of 2000, 1000, 500 and 250 μ M. Twenty μ l of bacterial inoculum was added to all the wells. Finally, 100 μ l of the suitable broth were added to each well. The optical density of the wells of the microtiter plate was determined using a microplate reader set at 550 nm and the plate was then incubated under strictly anaerobic conditions for 24 hours. Controls were made by replacing the peptide dilutions with PBS alone. After the incubation, the mixtures in each well were mixed manually to resuspend sedimented bacteria and the plate was read again. The biologic activity was calculated according to the formula:

$$100 - [((\text{Fin ODexp} - \text{In ODexp}) / (\text{Fin ODctr} - \text{In ODctr})) \times 100]$$

where:

Fin ODexp is the OD of the final experimental group;
In ODexp is the OD of the initial experimental group;
Fin ODctr is the OD of the final control group; and
In ODctr is the OD of the initial control group.

In addition, the % increase in time to reach mid-log phase growth was calculated.

The data presentation represent the means (\pm SEM) of at least 2 separate experiments.

-25-

D. INHIBITION OF HEMAGGLUTINATION ASSAYS(1) Strains and Growth Conditions for Hemagglutination Assays

5 The *P. gingivalis* strain of Section C.(1) was also used for the hemagglutination assays. The bacterial growth and culture conditions were also the same as those described in Section C.(1).

(2) Hemagglutination Assay

10 A classic assay was utilized to determine the hemagglutination potential of the *P. gingivalis* strain. Microorganisms were inoculated into BFB broth and cultured overnight, for approximately 24 hours at 37°C under strictly anaerobic conditions with continuous agitation on a minishaker (IKA-
15 Labortechnik, Staufen i. Br., Germany). The bacteria were harvested by centrifugation at 3,000 r.p.m. for 20 min, at 4°C, washed twice in 0.01 M phosphate buffered saline (PBS), pH 7.4, and suspended in the same buffer to an optical density of 1.0 at 550 nm. Erythrocytes
20 were obtained from a young male with O-type blood, since no difference in hemagglutination was observed in preliminary experiments with different ABO blood groups. One ml of blood was drawn each time, washed twice in PBS at 1,000 r.p.m. for 10 min at 4°C and
25 suspended in the same buffer at a 2% (v/v) final concentration. Fifty µl of the bacterial suspension were serially diluted in PBS (two-fold steps) in a 96-well U-bottom microplate (Costar, Cambridge, MA). Fifty µl of the erythrocyte suspension were added to
30 each well. Controls without bacteria or erythrocytes were included. The microplate was slightly shaken and incubated at room temperature for 2 hours. Visible examination on a white background was used to

-26-

determine hemagglutination. The amount of hemagglutination was rated as none (-), moderate (+/-), or strong (+). Erythrocytes in control wells with PBS precipitated to the center of the well, whereas erythrocyte-bacteria aggregates precipitated at the periphery of the bottom. The hemagglutination titer was expressed as the reciprocal of the highest dilution of the bacterial suspension providing visible hemagglutination.

10 (3) Histatin Peptide Inhibition of Hemagglutination Assay

Preparation of erythrocyte and bacterial suspensions were the same as for the hemagglutination assay. Fifty μ l of histatin peptide solutions were diluted in PBS in a U-bottom microplate, at various two-fold concentrations with 600 nmole/ml being the highest. The bacterial concentration utilized was normally twice the minimal concentration which gave strong hemagglutination. Equal volumes of the bacterial suspension were poured into the wells containing the histatin peptides. Finally, 50 μ l of erythrocyte suspension were added in each well. The microplate was slightly shaken and incubated at room temperature for 2 hours. Controls were made by replacement of the peptide dilutions with PBS only. The experiments were conducted at least twice. The lowest histatin peptide concentration without hemagglutination (complete inhibition) was determined upon visual examination. The highest final histatin peptide concentration utilized was 100 nmole/ml.

E. CLOSTRIPAIN ASSAYS

Clostripain from *Clostridium histolyticum* (Sigma Chemical Corp., St. Louis, MO) was dissolved in deionized

-27-

water to a concentration of 1 mg/mL (300 units/mg) and activated with the addition of 10 mmol/L DTT. To measure its hydrolytic activity, clostripain (6 units) was added to 50 nmol/L Hepes buffer, pH 7.5, containing 80 μ mol/L BAPNA (benzoyl-arginine-p-nitroanilide), together with 5.6 μ mol/L of histatin peptide inhibitor. As controls, assays were performed in the absence of any histatin peptide inhibitor. The activity was monitored continuously at 405 nm using a Molecular Devices V_{Max} microtitre plate reader. The activities were determined from the maximum rates of substrate hydrolysis. Assays were done in duplicate, and the means normalized to the controls.

EXAMPLE 2. EFFECTS OF HISTATIN PEPTIDES ON FUNGAL OR BACTERIAL VIABILITY

Figures 2-11 and Table 1 summarize the results of the fungal killing, bacterial growth inhibition, bacterial cell killing, bacteria mediated hemagglutination inhibition and bacterial enzyme (clostripain) inhibition effects of histatin 5 and several tested histatin peptides. For comparison purposes, the anti-fungal and anti-bacterial effects of histatin 11 and the histatin-based peptides other than amino acid substituted variants of peptide 113 were assessed with synthesized histatin 5 as a standard. The amino acid substituted variants of peptide 113 were assessed for their anti-fungal and anti-bacterial effects with peptide 113 as a standard.

Histatin 11 and histatin-based peptides 113, 117, 118, 119, 120 and 129 have *C. albicans* blastoconidia killing, *P. gingivalis* growth inhibition, bacteria mediated hemagglutination inhibition and clostripain inhibition effects. The various modified peptide 113 variants, i.e. peptide 113-F4, peptide 113-F5, peptide 113-F12, peptide 113-F4.5, peptide 113-F4.5.12, peptide 113-K6, peptide 113-

-28-

H8, peptide 113-K6H8, peptide 113-F8, peptide 113-L4.5.12, peptide 113-Y4.5.12, peptide 113-Q2.10, peptide 113-Q3.9, peptide 113 with an acetyl blocking group on the N-terminus of the peptide, and peptide 113 with a carbamyl blocking group on the N-terminus also exhibit anti-fungal and anti-bacterial activity. These antimicrobial effects are similar to those observed for histatin 5 and for histatin-based peptides 101-105. Although expected variations exist in anti-fungal and anti-bacterial effects between the tested peptides, the antimicrobial effects of the histatin-based peptides are comparable to those of histatin 5. These results demonstrate that these histatin-based peptides are efficacious as anti-fungal or anti-bacterial agents. In particular, these results demonstrate that histatin-based peptide 113 and its subpeptides histatin 11, histatin-based peptides 117, 118, 119, 120 and 129 are more efficacious on the basis of molecular weight or amino acid sequence length than histatin 5. The anti-fungal and anti-bacterial activity of the histatins appears to be concentrated in the amino acid sequence of histatin-based peptide 113. Selected amino acid substitutions to form variants of peptide 113 also retain and often enhance the anti-fungal and anti-bacterial activity of the original peptide.

-29-

TABLE 1

SUMMARY OF HISTATIN PEPTIDE SEQUENCES
AND BIOLOGICAL ACTIVITY TESTING

<u>Histatin Peptide</u>	<u>MW</u>	<u>C. albicans</u>	<u>P. gingivalis</u>	<u>Hemagglut</u>	<u>Clostripain</u>
SynHis5	3037	3.0 μ M	88.7%	3.12 μ M	<6.2 μ M
101	2025	3.5		1.56	7.5
102		10	+++		
103	2613	4.5	56.6	1.56	19
104	2055	NA			
105	2978	5.0	+++	6.25	3.3
His 11	1080	+		100	NA
113	1563	5.8	34.95	25	25
117	1492	++++			
118	1426	++++		6.25	45
119	1279	++		25	>50
120	1151	++		50	>50
129		27	15.4	50	19

Key:

C. albicans: Results expressed as LD₅₀ (μ M) for % killing or % killing at 50 μ M dose (see below for scoring).

P. gingivalis: Results expressed as % increase in time to reach mid log-phase growth (0.2 OD) for the 2 mM dose or % inhibition at 500 μ M dose (see below for scoring).

Hemagglutination: Results expressed as lowest dose (μ M) at which hemagglutination was observed using *P. Gingivalis*.

Clostripain: Results expressed as IC₅₀ (μ M) for inhibition of Clostripain.

Scoring: The highest response seen was used in the scoring.

++++	80 - 100%
+++	50 - 80%
++	20 - 50%
+	<20%
NA	Not Active

-30-

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention
5 described herein. Such equivalents are intended to be encompassed by the following claims.

-31-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Periodontix, Inc.
(B) STREET: 313 Pleasant Street
(C) CITY: Watertown
(D) STATE/PROVINCE: MA
(F) COUNTRY: USA
(G) POSTAL CODE/ZIP 02171
(H) TELEPHONE: (617) 926-1980
(I) TELEFAX (617) 926-4776

(i) APPLICANT:

(A) NAME: Trustees of Boston University
(B) STREET: 147 Bay State Road
(C) CITY: Boston
(D) STATE/PROVINCE: MA
(F) COUNTRY: USA
(G) POSTAL CODE/ZIP 02215
(H) TELEPHONE: (617) 638-4540
(I) TELEFAX (617) 638-4515

(i) APPLICANT/INVENTOR:

(A) NAME: Frank G. Oppenheim
(B) STREET: 10 Heath Wood Lane
(C) CITY: Chestnut Hill
(D) STATE/PROVINCE: MA
(E) COUNTRY: USA
(F) POSTAL CODE/ZIP: 02167

(i) APPLICANT/INVENTOR:

(A) NAME: Tao Xu
(B) STREET: 2 Bridle Path Circle
(C) CITY: Newton
(D) STATE/PROVINCE: MA
(E) COUNTRY: USA
(F) POSTAL CODE/ZIP: 02166

(i) APPLICANT/INVENTOR

(A) NAME: Peter Spacchiapoli
(B) STREET: 9 High Road
(C) CITY: Newbury
(D) STATE/PROVINCE: MA
(E) COUNTRY: USA
(F) POSTAL CODE/ZIP: 01951

(i) APPLICANT/INVENTOR

(A) NAME: F. Donald Roberts
(B) STREET: 2 Bridle Path Circle
(C) CITY: Dover
(D) STATE/PROVINCE: MA
(E) COUNTRY: USA
(F) POSTAL CODE/ZIP: 02030

(i) APPLICANT/INVENTOR

(A) NAME: Phillip M. Friden
(B) STREET: 32 Washington Street
(C) CITY: Bedford
(D) STATE/PROVINCE: MA

-32-

(E) COUNTRY: USA
(F) POSTAL CODE/ZIP: 01730

(ii) TITLE OF INVENTION: Anti-Fungal D-Amino Acid Histatin-Based Peptides

(iii) NUMBER OF SEQUENCES: 37

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Hamilton, Brook, Smith & Reynolds, P.C.
(B) STREET: Two Militia Drive
(C) CITY: Lexington
(D) STATE: MA
(E) COUNTRY: US
(F) ZIP: 02173

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/485,273
(B) FILING DATE: 07-JUN-1995

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Brook, David E.
(B) REGISTRATION NUMBER: 22,592
(C) REFERENCE/DOCKET NUMBER: PER95-02A2 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-861-6240
(B) TELEFAX: 617-861-9540

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /product= "PSE"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 1..38
(D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

-33-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asp Xaa His Glu Lys Arg His His Gly Tyr Arg Arg Lys Phe His Glu
 1 5 10 15
 Lys His His Ser His Arg Glu Phe Pro Phe Tyr Gly Asp Tyr Gly Ser
 20 25 30
 Asn Tyr Leu Tyr Asp Asn
 35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..27
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Arg Lys Phe His Glu Lys His His Ser His Arg Glu Phe Pro Phe Tyr
 1 5 10 15
 Gly Asp Tyr Gly Ser Asn Tyr Leu Tyr Asp Asn
 20 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..32
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Ser His Ala Lys Arg His His Gly Tyr Lys Arg Lys Phe His Glu
 1 5 10 15
 Lys His His Ser His Arg Gly Tyr Arg Ser Asn Tyr Leu Tyr Asp Asn
 20 25 30

-34-

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
 (B) LOCATION: 1..21
 (D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Lys Phe His Glu Lys His His Ser His Arg Gly Tyr Arg Ser Asn
1 5 10 15

Tyr Leu Tyr Asp Asn
 20

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
 (B) LOCATION: 1..24
 (D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Ser His Ala Lys Arg His His Gly Tyr Lys Arg Lys Phe His Glu
1 5 10 15

Lys His His Ser His Arg Gly Tyr
 20

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

-35-

(ix) FEATURE:
 (A) NAME/KEY: Region
 (B) LOCATION: 1..25
 (D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp Ser His Ala Lys Arg His His Gly Tyr Lys Arg Lys Phe His Glu
1 5 10 15
Lys His His Ser His Arg Gly Tyr Arg
 20 25

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Region
 (B) LOCATION: 1..13
 (D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Arg Lys Phe His Glu Lys His His Ser His Arg Gly Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 12 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Region
 (B) LOCATION: 1..12
 (D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Phe His Glu Lys His His Ser His Arg Gly Tyr
1 5 10

-36-

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..14
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Lys Phe His Glu Lys His His Ser His Arg Gly Tyr Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..13
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Phe His Glu Lys His His Ser His Arg Gly Tyr Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..8
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

-37-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Lys Arg His His Gly Tyr Lys Arg
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..7
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Lys Arg His His Gly Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..21
- (D) OTHER INFORMATION: /note= "At least one amino must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Lys Arg His His Gly Tyr Lys Arg Lys Phe His Glu Lys His His Ser
1 5 10 15

His Arg Gly Tyr Arg
20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

-38-

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Region
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Tyr Lys Arg Lys Phe His Glu Lys His His Ser His Arg Gly Tyr
1 5 10 15

Arg

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Region
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Lys Arg His His Gly Tyr Lys Arg Lys Phe His Glu Lys His His Ser
1 5 10 15

His Arg

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Region
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

-39-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gly Tyr Lys Arg Lys Phe His Glu Lys His His Ser His Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..15
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Lys Arg His His Gly Tyr Lys Arg Lys Phe His Glu Lys His His
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ala Lys Arg His His Gly Tyr Lys Arg Lys Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

-40-

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..11
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Lys Arg His His Gly Tyr Lys Arg Lys Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..11
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Lys Arg His His Gly Tyr Lys Arg Lys Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..10
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ala Lys Arg His His Gly Tyr Lys Arg Lys
1 5 10

-41-

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..9
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ala Lys Arg His His Gly Tyr Lys Arg
1 5

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..10
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Arg His His Gly Tyr Lys Arg Lys Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

-42-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ala Lys Arg Phe His Gly Tyr Lys Arg Lys Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala Lys Arg His Phe Gly Tyr Lys Arg Lys Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ala Lys Arg His His Gly Tyr Lys Arg Lys Phe Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

-43-

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ala Lys Arg Phe Phe Gly Tyr Lys Arg Lys Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Lys Arg Phe Phe Gly Tyr Lys Arg Lys Phe Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala Lys Arg His His Lys Tyr Lys Arg Lys Phe His
1 5 10

-44-

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ala Lys Arg His His Gly Tyr His Arg Lys Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ala Lys Arg His His Lys Tyr His Arg Lys Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..12
- (D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

-45-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ala Lys Arg His His Gly Tyr Phe Arg Lys Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 1..12

(D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ala Lys Arg Leu Leu Gly Tyr Lys Arg Lys Phe Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 1..12

(D) OTHER INFORMATION: /note= "At least one amino acid must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Ala Lys Arg Tyr Tyr Gly Tyr Lys Arg Lys Phe Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

-46-

(ix) FEATURE:
 (A) NAME/KEY: Region
 (B) LOCATION: 1..12
 (D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Ala Gln Arg His His Gly Tyr Lys Arg Gln Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 12 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Region
 (B) LOCATION: 1..12
 (D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Ala Lys Gln His His Gly Tyr Lys Gln Lys Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 12 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
 (A) NAME/KEY: Region
 (B) LOCATION: 1..12
 (D) OTHER INFORMATION: /note= "At least one amino acid
must have a D configuration."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ala Gln Gln His His Gly Tyr Lys Gln Gln Phe His
1 5 10

-47-

CLAIMS

1. A composition for treating a fungal or bacterial infection comprising a substantially pure peptide having an amino acid sequence, of at least 8 amino acids, selected from the group consisting of:
- 5
- a) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18;
- b) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where the glycine at position 6 is replaced by lysine, arginine or another basic amino acid;
- 10
- c) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where the lysine at position 8 is replaced by histidine, phenylalanine or another hydrophobic amino acid;
- 15
- d) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where at least one of the histidines at positions 4, 5 and 12 is replaced by phenylalanine, tyrosine, leucine or another hydrophobic amino acid;
- 20
- e) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where at least one of the lysines at positions 2 and 10 is replaced by glutamine, arginine or by another basic amino acid;
- 25
- f) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where at least one of the arginines at positions 3 and 9 is replaced by glutamine, lysine or by another basic amino acid; and
- 30
- g) any combination of the amino acid replacements of preceding sections b) - f) with the exception

-48-

that glutamine or any other non-basic amino acid cannot simultaneously occupy positions 2, 3, 9 and 10 of the amino acid sequence.

2. A composition of Claim 1 wherein said substantially
5 pure peptide is selected from the group consisting of:
- a) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18;
 - b) the amino acid sequence of histatin 11 as set forth in SEQ ID NO: 11;
 - 10 c) the amino acid sequence of peptide 129 as set forth in SEQ ID NO: 23;
 - d) the amino acid sequence of peptide 117 as set forth in SEQ ID NO: 19;
 - e) the amino acid sequence of peptide 118 as set forth in SEQ ID NO: 20;
 - 15 f) the amino acid sequence of peptide 119 as set forth in SEQ ID NO: 21;
 - g) the amino acid sequence of peptide 120 as set forth in SEQ ID NO: 22;
 - 20 h) the amino acid sequence of peptide 113-F4 as set forth in SEQ ID NO: 24;
 - i) the amino acid sequence of peptide 113-F5 as set forth in SEQ ID NO: 25;
 - j) the amino acid sequence of peptide 113-F12 as set forth in SEQ ID NO: 26;
 - 25 k) the amino acid sequence of peptide 113-F4.5 as set forth in SEQ ID NO: 27;
 - l) the amino acid sequence of peptide 113-F4.5.12 as set forth in SEQ ID NO: 28;
 - 30 m) the amino acid sequence of peptide 113-K6 as set forth in SEQ ID NO: 29;
 - n) the amino acid sequence of peptide 113-H8 as set forth in SEQ ID NO: 30;

-49-

- o) the amino acid sequence of peptide 113-K6H8 as set forth in SEQ ID NO: 31;
- p) the amino acid sequence of peptide 113-F8 as set forth in SEQ ID NO: 32;
- 5 q) the amino acid sequence of peptide 113-L4.5.12 as set forth in SEQ ID NO: 33;
- r) the amino acid sequence of peptide 113-Y4.5.12 as set forth in SEQ ID NO: 34;
- s) the amino acid sequence of peptide 113-Q2.10 as set forth in SEQ ID NO: 35;
- 10 t) the amino acid sequence of peptide 113-Q3.9 as set forth in SEQ ID NO: 36; and
- u) combinations of two or more of the above with the exception of peptide 113-Q2.3.9.10 as set forth in SEQ ID NO: 37.
- 15
3. A composition of Claim 1 wherein the peptide has a modification comprising a substituent addition to either the N-terminus, the C-terminus, or to both the N-terminus and C-terminus of said peptide.
- 20 4. A composition of Claim 3 wherein the peptide has at least one of said modifications selected from the group consisting of:
- a) an acetyl or a carbamyl addition at the N-terminus; and
- 25 b) an amide addition at the C-terminus.
5. Substantially pure peptide having an amino acid sequence, of at least 8 amino acids, selected from the group consisting of:
- 30 a) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18.
- b) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where the glycine at

-50-

- position 6 is replaced by lysine, arginine or another basic amino acid;
- 5 c) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where the lysine at position 8 is replaced by histidine, phenylalanine or another hydrophobic amino acid;
- 10 d) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where at least one of the histidines at positions 4, 5 and 12 is replaced by phenylalanine, tyrosine, leucine or another hydrophobic amino acid;
- 15 e) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where at least one of the lysines at positions 2 and 10 is replaced by glutamine, arginine or by another basic amino acid;
- 20 f) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where at least one of the arginines at positions 3 and 9 is replaced by glutamine, lysine or by another basic amino acid; and
- 25 g) any combination of the amino acid replacements of preceding sections b) - f) with the exception that glutamine or any other non-basic amino acid cannot simultaneously occupy positions 2, 3, 9 and 10 of the amino acid sequence.
6. Substantially pure peptide of Claim 5 selected from the group consisting of:
- 30 a) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18;
- b) the amino acid sequence of histidine 11 as set forth in SEQ ID NO: 11;
- c) the amino acid sequence of peptide 129 as set forth in SEQ ID NO: 23;

-51-

- d) the amino acid sequence of peptide 117 as set forth in SEQ ID NO: 19;
- e) the amino acid sequence of peptide 118 as set forth in SEQ ID NO: 20;
- 5 f) the amino acid sequence of peptide 119 as set forth in SEQ ID NO: 21;
- g) the amino acid sequence of peptide 120 as set forth in SEQ ID NO: 22;
- 10 h) the amino acid sequence of peptide 113-F4 as set forth in SEQ ID NO: 24;
- i) the amino acid sequence of peptide 113-F5 as set forth in SEQ ID NO: 25;
- j) the amino acid sequence of peptide 113-F12 as set forth in SEQ ID NO: 26;
- 15 k) the amino acid sequence of peptide 113-F4.5 as set forth in SEQ ID NO: 27;
- l) the amino acid sequence of peptide 113-F4.5.12 as set forth in SEQ ID NO: 28;
- 20 m) the amino acid sequence of peptide 113-K6 as set forth in SEQ ID NO: 29;
- n) the amino acid sequence of peptide 113-H8 as set forth in SEQ ID NO: 30;
- o) the amino acid sequence of peptide 113-K6H8 as set forth in SEQ ID NO: 31;
- 25 p) the amino acid sequence of peptide 113-F8 as set forth in SEQ ID NO: 32;
- q) the amino acid sequence of peptide 113-L4.5.12 as set forth in SEQ ID NO: 33;
- r) the amino acid sequence of peptide 113-Y4.5.12 as set forth in SEQ ID NO: 34;
- 30 s) the amino acid sequence of peptide 113-Q2.10 as set forth in SEQ ID NO: 35; and
- t) the amino acid sequence of peptide 113-Q3.9 as set forth in SEQ ID NO: 36.

-52-

7. A substantially pure peptide of Claim 5 wherein the peptide has a modification comprising a substituent addition to either the N-terminus, the C-terminus, or to both the N-terminus and C-terminus of said peptide.
- 5 8. A substantially pure peptide of Claim 7 wherein the peptide has at least one of said modifications selected from the group consisting of:
- a) an acetyl or a carbamyl addition at the N-terminus; and
 - 10 b) an amide addition at the C-terminus.
9. A method for treating a fungal or bacterial infection in an individual comprising administering to said individual a therapeutically effective amount of at least one peptide having an amino acid sequence, of at
- 15 least 8 amino acids, selected from the group consisting of:
- a) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18;
 - 20 b) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where the glycine at position 6 is replaced by lysine, arginine or another basic amino acid;
 - c) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where the lysine at
 - 25 position 8 is replaced by histidine, phenylalanine or another hydrophobic amino acid;
 - d) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where at least one of the histidines at positions 4, 5 and 12 is replaced
 - 30 by phenylalanine, tyrosine, leucine or another hydrophobic amino acid;
 - e) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where at least one of the

-53-

lysines at positions 2 and 10 is replaced by glutamine, arginine or by another basic amino acid;

- 5 f) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18 where at least one of the arginines at positions 3 and 9 is replaced by glutamine, lysine or by another basic amino acid;
- 10 g) any combination of the amino acid replacements of preceding sections b) - f) with the exception that glutamine or any other non-basic amino acid cannot simultaneously occupy positions 2, 3, 9 and 10 of the amino acid sequence.
- 15 10. A method for treating a fungal or bacterial infection of Claim 9 wherein said peptide is selected from the group consisting of:
- 20 a) the amino acid sequence of peptide 113 as set forth in SEQ ID NO: 18;
- b) the amino acid sequence of histatin 11 as set forth in SEQ ID NO: 11;
- 25 c) the amino acid sequence of peptide 129 as set forth in SEQ ID NO: 23;
- d) the amino acid sequence of peptide 117 as set forth in SEQ ID NO: 19;
- e) the amino acid sequence of peptide 118 as set forth in SEQ ID NO: 20;
- 30 f) the amino acid sequence of peptide 119 as set forth in SEQ ID NO: 21;
- g) the amino acid sequence of peptide 120 as set forth in SEQ ID NO: 22.
- h) the amino acid sequence of peptide 113-F4 as set forth in SEQ ID NO: 24;
- i) the amino acid sequence of peptide 113-F5 as set forth in SEQ ID NO: 25;

-54-

- j) the amino acid sequence of peptide 113-F12 as set forth in SEQ ID NO: 26;
- k) the amino acid sequence of peptide 113-F4.5 as set forth in SEQ ID NO: 27;
- 5 l) the amino acid sequence of peptide 113-F4.5.12 as set forth in SEQ ID NO: 28;
- m) the amino acid sequence of peptide 113-K6 as set forth in SEQ ID NO: 29;
- 10 n) the amino acid sequence of peptide 113-H8 as set forth in SEQ ID NO: 30;
- o) the amino acid sequence of peptide 113-K6H8 as set forth in SEQ ID NO: 31;
- p) the amino acid sequence of peptide 113-F8 as set forth in SEQ ID NO: 32;
- 15 q) the amino acid sequence of peptide 113-L4.5.12 as set forth in SEQ ID NO: 33;
- r) the amino acid sequence of peptide 113-Y4.5.12 as set forth in SEQ ID NO: 34;
- s) the amino acid sequence of peptide 113-Q2.10 as set forth in SEQ ID NO: 35; and
- 20 t) the amino acid sequence of peptide 113-Q3.9 as set forth in SEQ ID NO: 36.
11. A method for treating a fungal or bacterial infection of Claim 9 wherein the peptide has a modification
- 25 comprising a substituent addition to either the N-terminus, the C-terminus, or to both the N-terminus and C-terminus of said peptide.
12. A method for treating a fungal or bacterial infection of Claim 11 wherein the peptide has at least one of
- 30 said modifications selected from the group consisting of:
- a) an acetyl or a carbamyl addition at the N-terminus; and

-55-

- b) an amide addition at the C-terminus.
13. A method for treating a fungal or bacterial infection of Claim 9 wherein said fungal or bacterial infection is selected from the group consisting of:
- 5 a) an infection of the oral cavity;
b) an infection of the vagina;
c) an infection of the urethra;
d) an infection of the ear;
e) an infection of the skin;
10 f) a respiratory infection;
g) a mucosal infection;
h) an ophthalmic infection; and
i) a systemic infection.
14. A method for treating a fungal or bacterial infection of Claim 13 wherein the fungus or bacterium is selected from the group consisting of:
- 15 a) *Candida albicans*;
b) *Actinomyces actinomycetemcomitans*;
c) *Actinomyces viscosus*;
20 d) *Bacteroides forsythus*;
e) *Bacteriodes fragilis*;
f) *Bacteriodes gracilis*;
g) *Bacteriodes ureolyticus*;
h) *Campylobacter concisus*;
25 i) *Campylobacter rectus*;
j) *Campylobacter showae*;
k) *Campylobacter sputorum*;
l) *Capnocytophaga gingivalis*;
m) *Capnocytophaga ochracea*;
30 n) *Capnocytophaga sputigena*;
o) *Clostridium histolyticum*;
p) *Eikenella corrodens*;
q) *Eubacterium nodatum*;

-56-

- r) *Fusobacterium nucleatum*;
- s) *Fusobacterium periodonticum*;
- t) *Peptostreptococcus micros*;
- u) *Porphyromonas endodontalis*;
- 5 v) *Porphyromonas gingivalis*;
- w) *Prevotella intermedia*;
- x) *Prevotella nigrescens*;
- y) *Propionibacterium acnes*;
- z) *Pseudomonas aeruginosa*;
- 10 aa) *Selenomonas noxia*;
- bb) *Staphylococcus aureus*;
- cc) *Streptococcus constellatus*;
- dd) *Streptococcus gordonii*;
- ee) *Streptococcus intermedius*;
- 15 ff) *Streptococcus mutans*;
- gg) *Streptococcus oralis*;
- hh) *Streptococcus pneumonia*;
- ii) *Streptococcus sanguis*;
- kk) *Treponema denticola*;
- 20 ll) *Treponema pectinovorum*;
- mm) *Treponema socranskii*;
- nn) *Veillonella parvula*; and
- oo) *Wolinella succinogenes*.

1/14

Histatin 1:	1	5	10	15	Asp-Pse-His-Glu-Lys-Arg-His-His-Gly-Tyr-Arg-Arg-Lys-Phe-His-Glu-Lys-His-His-
Histatin 2:					Arg-Lys-Phe-His-Glu-Lys-His-His-
Histatin 3:	1	5	10	15	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-
Histatin 4:					Arg-Lys-Phe-His-Glu-Lys-His-His-
Histatin 5:					Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-
Histatin 6:					Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-
Histatin 7:					Arg-Lys-Phe-His-Glu-Lys-His-His-
Histatin 8:					Lys-Phe-His-Glu-Lys-His-His-
Histatin 9:					Arg-Lys-Phe-His-Glu-Lys-His-His-
Histatin 10:					Lys-Phe-His-Glu-Lys-His-His-
Histatin 11:					Lys-Arg-His-His-Gly-Tyr-Lys-Arg
Histatin 12:					Lys-Arg-His-His-Gly-Tyr-Lys
Peptide 101:					Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-
Peptide 102:					Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-
Peptide 103:					Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-

FIGURE 1A

2/14

Peptide 104:	10	15
Peptide 105:		Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-
Peptide 113:		Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His
Peptide 113-F4		Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His
Peptide 113-F5		Ala-Lys-Arg-Phe-His-Gly-Tyr-Lys-Arg-Lys-Phe-His
Peptide 113-F12		Ala-Lys-Arg-His-Phe-Gly-Tyr-Lys-Arg-Lys-Phe-His
Peptide 113-F4.5		Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-Phe
Peptide 113-F4.5.12		Ala-Lys-Arg-Phe-Phe-Gly-Tyr-Lys-Arg-Lys-Phe-His
Peptide 113-K6		Ala-Lys-Arg-Phe-Phe-Gly-Tyr-Lys-Arg-Lys-Phe-Phe
Peptide 113-H8		Ala-Lys-Arg-His-His-Lys-Tyr-Lys-Arg-Lys-Phe-His
Peptide 113-K6H8		Ala-Lys-Arg-His-His-Gly-Tyr-His-Arg-Lys-Phe-His
Peptide 113-F8		Ala-Lys-Arg-His-His-Lys-Tyr-His-Arg-Lys-Phe-His
Peptide 113-L4.5.12		Ala-Lys-Arg-His-His-Gly-Tyr-Phe-Arg-Lys-Phe-His
Peptide 113-Y4.5.12		Ala-Lys-Arg-Leu-Leu-Gly-Tyr-Lys-Arg-Lys-Phe-Leu
Peptide 113-Q2.10		Ala-Lys-Arg-Tyr-Tyr-Gly-Tyr-Lys-Arg-Lys-Phe-Tyr
		Ala-Gln-Arg-His-His-Gly-Tyr-Lys-Arg-Gln-Phe-His

FIGURE 1B

Peptide 113-Q3.9	5	10	15
	Ala-Lys-Gln-His-His-Gly-Tyr-Lys-Gln-Lys-Phe-His		
Peptide 113-Q2.3.9.10			
	Ala-Gln-Gln-His-His-Gly-Tyr-Lys-Gln-Gln-Phe-His		
Peptide 117:			
	Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His		
Peptide 118:			
	Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe		
Peptide 119:			
	Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys		
Peptide 120:			
	Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg		
Peptide 129:			
	Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe		
Histatin 1:	20	25	30
	Ser-His-Arg-Glu-Phe-Pro-Phe-Tyr-Gly-Asp-Tyr-Gly-Ser-Asn-Tyr-Leu-Tyr-Asp-Asn		
Histatin 2:			
	Ser-His-Arg-Glu-Phe-Pro-Phe-Tyr-Gly-Asp-Tyr-Gly-Ser-Asn-Tyr-Leu-Tyr-Asp-Asn		
Histatin 3:			
	Ser-His-Arg-Gly-Tyr-Arg-Ser-Asn-Tyr-Leu-Tyr-Asp-Asn		
Histatin 4:			
	Ser-His-Arg-Gly-Tyr-Arg-Ser-Asn-Tyr-Leu-Tyr-Asp-Asn		
Histatin 5:			
	Ser-His-Arg-Gly-Tyr		
Histatin 6:			
	Ser-His-Arg-Gly-Tyr-Arg		
Histatin 7:			
	Ser-His-Arg-Gly-Tyr		
Histatin 8:			
	Ser-His-Arg-Gly-Tyr		

FIGURE 1C

	20	25
Histatin 9:	Ser-His-Arg-Gly-Tyr-Arg	
Histatin 10:	Ser-His-Arg-Gly-Tyr-Arg	
Peptide 101:	Ser-His-Arg-Gly-Tyr-Arg	
Peptide 102:	Ser-His-Arg-Gly-Tyr-Arg	
Peptide 103:	Ser-His-Arg	
Peptide 104:	Ser-His-Arg	

FIGURE 1D

5/14

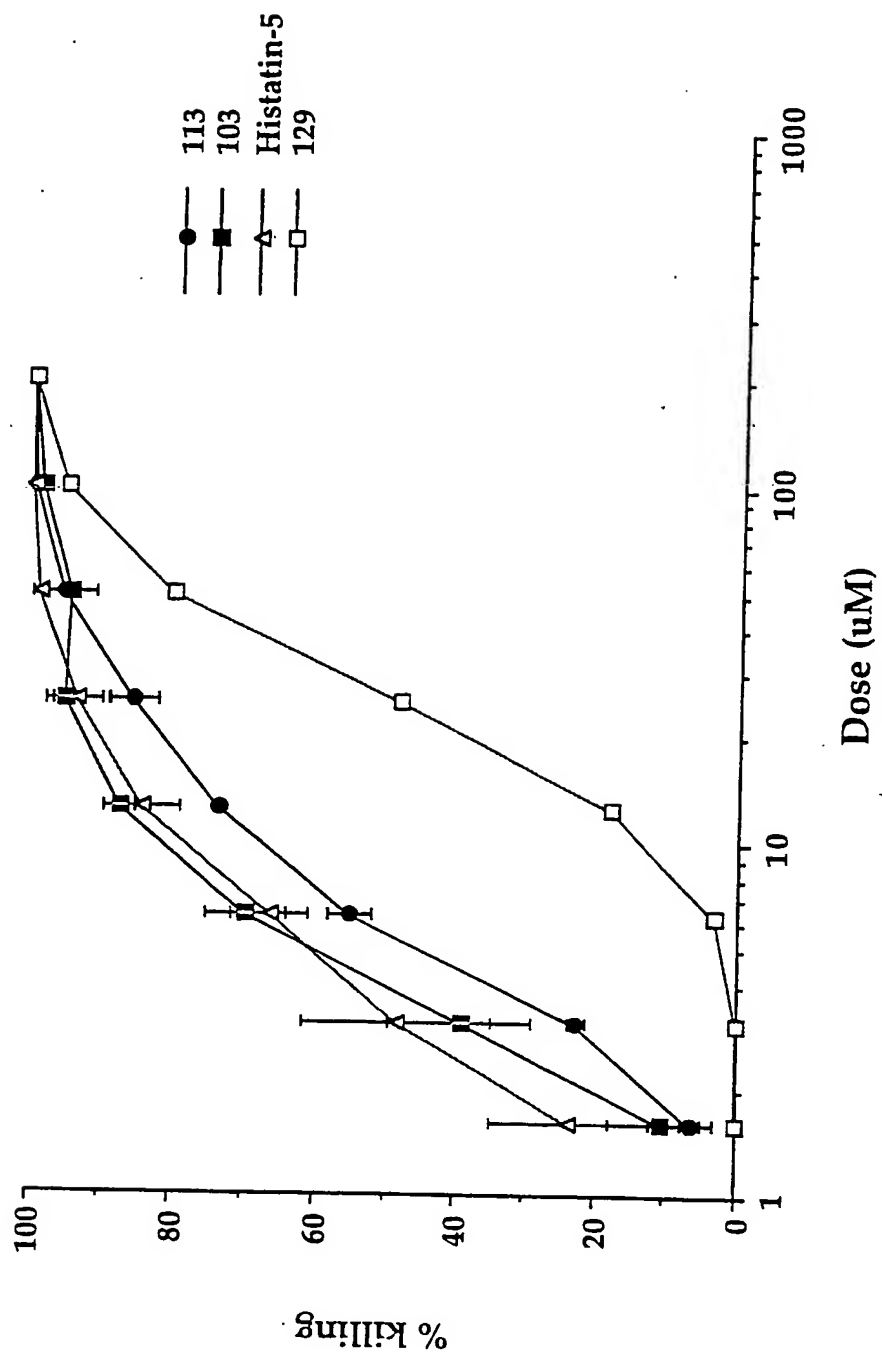


FIGURE 2

6/14

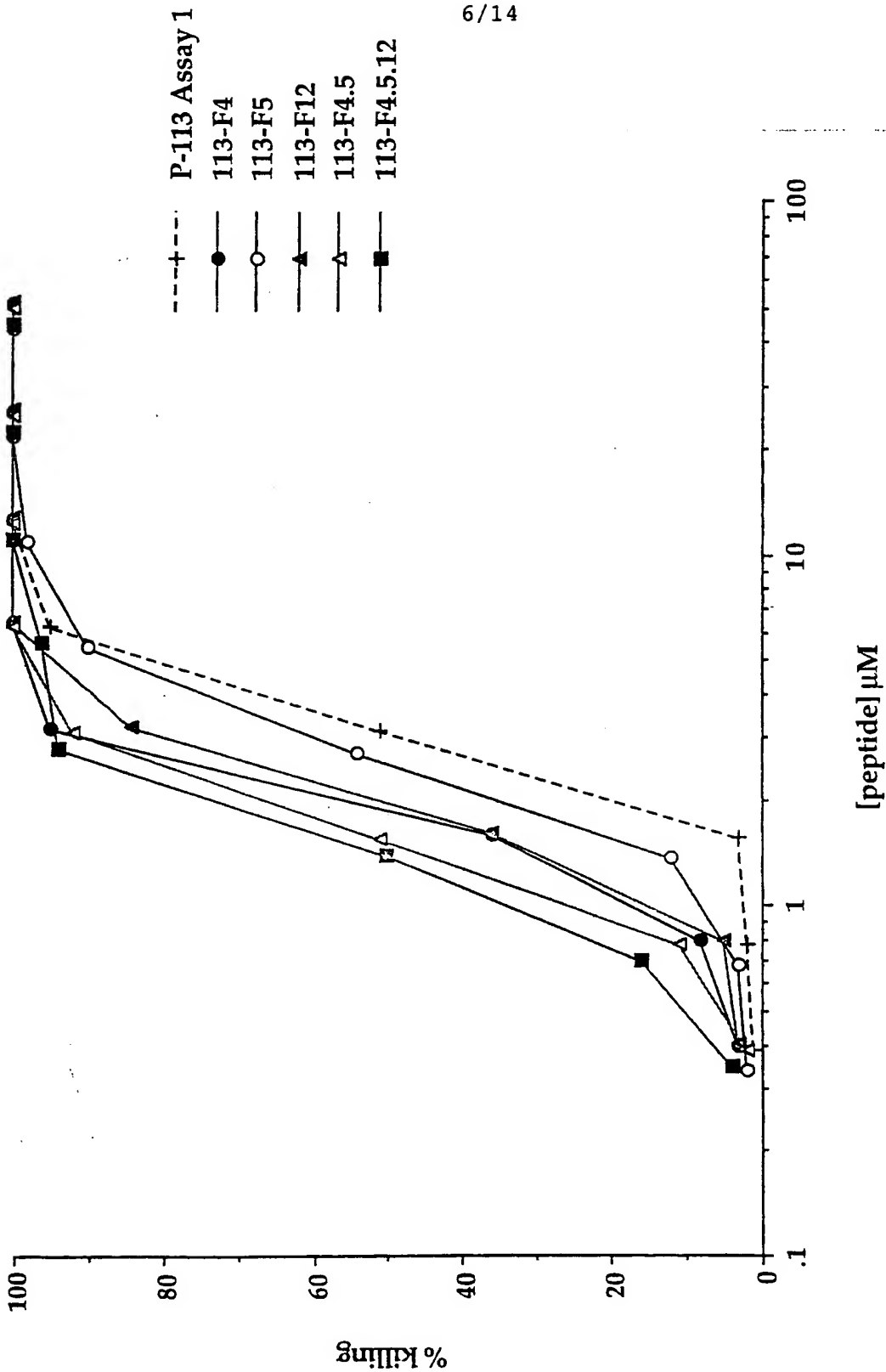


FIGURE 3

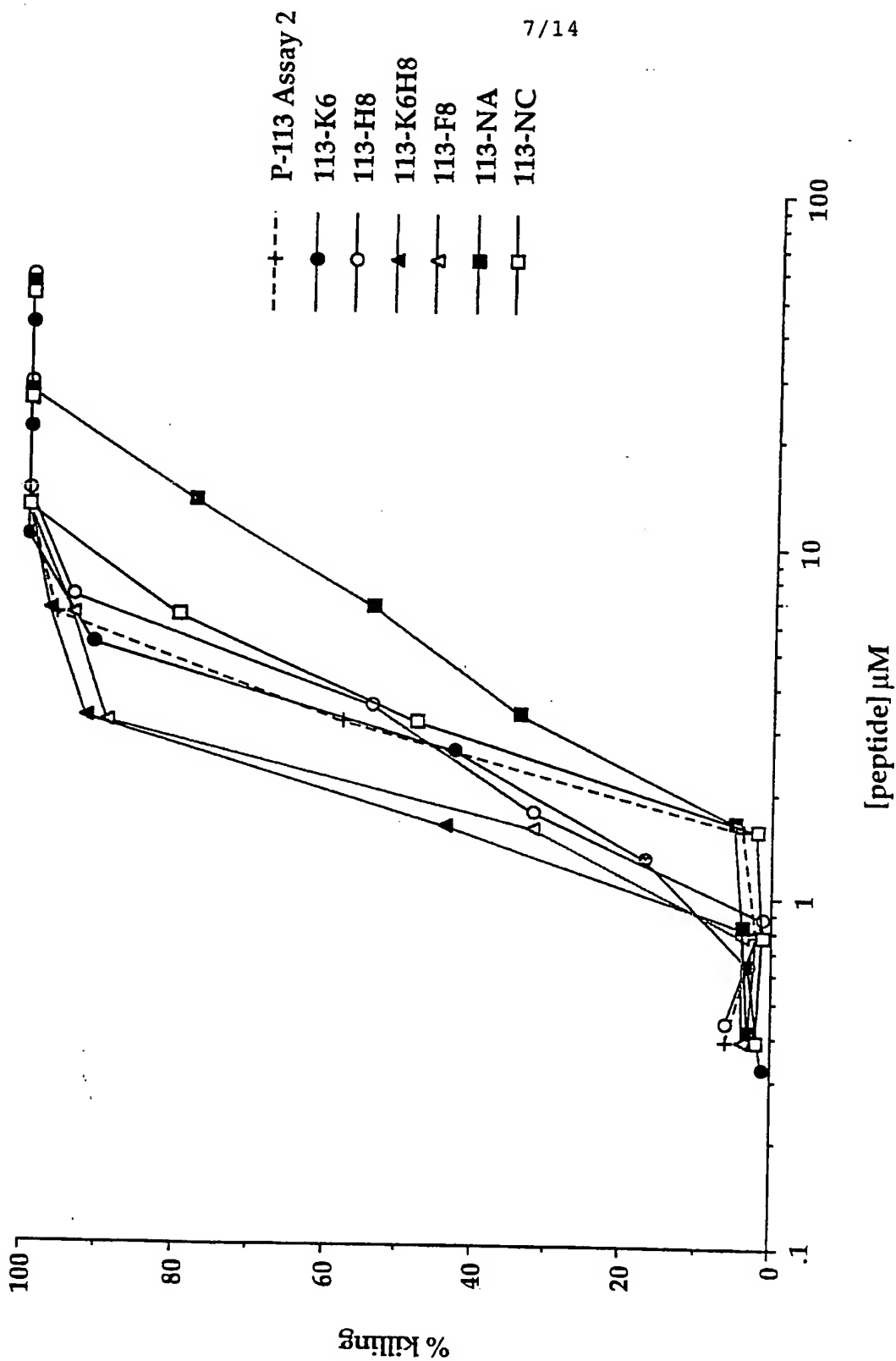
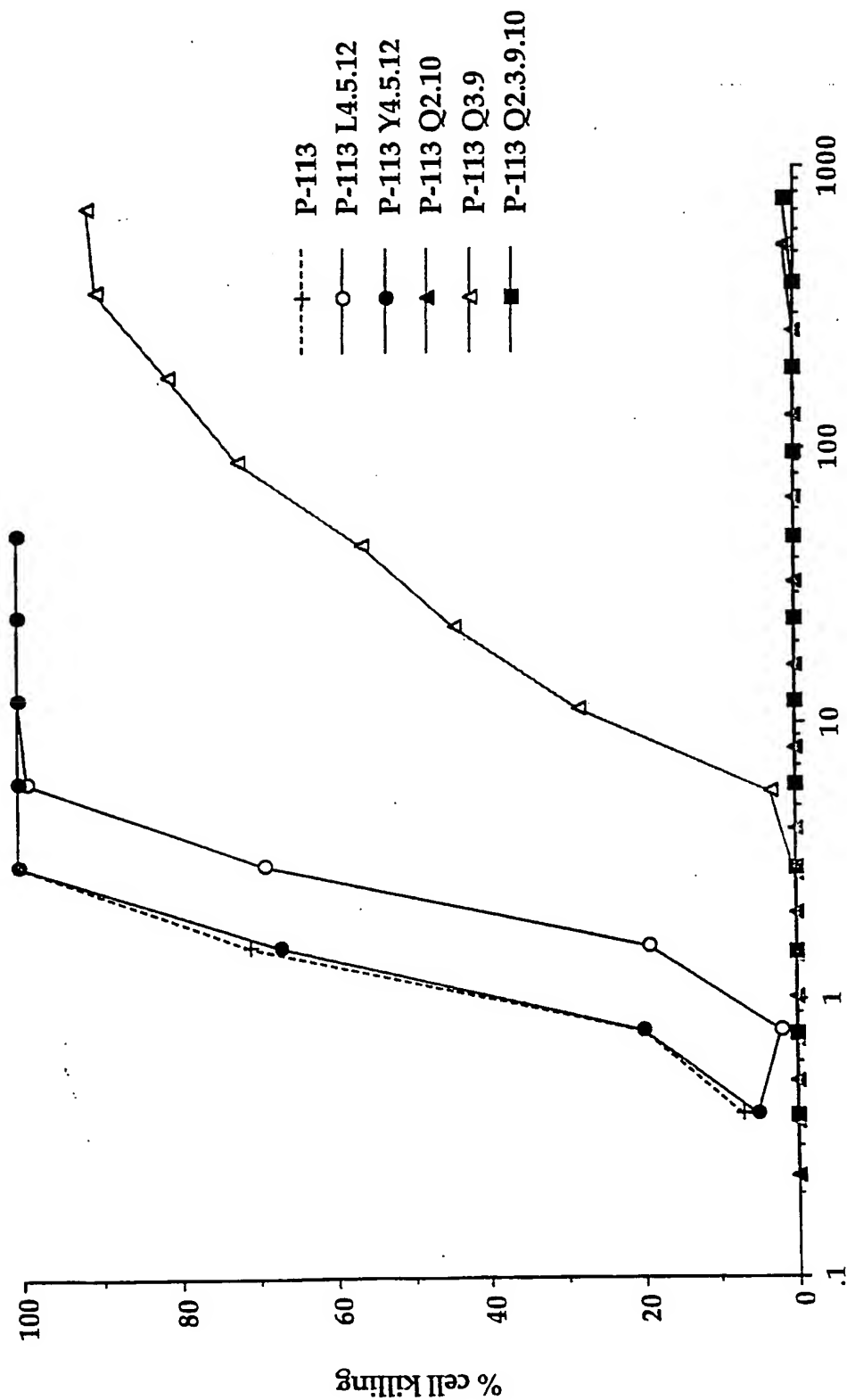


FIGURE 4

8/14



[peptidel μM]

FIGURE 5

9/14

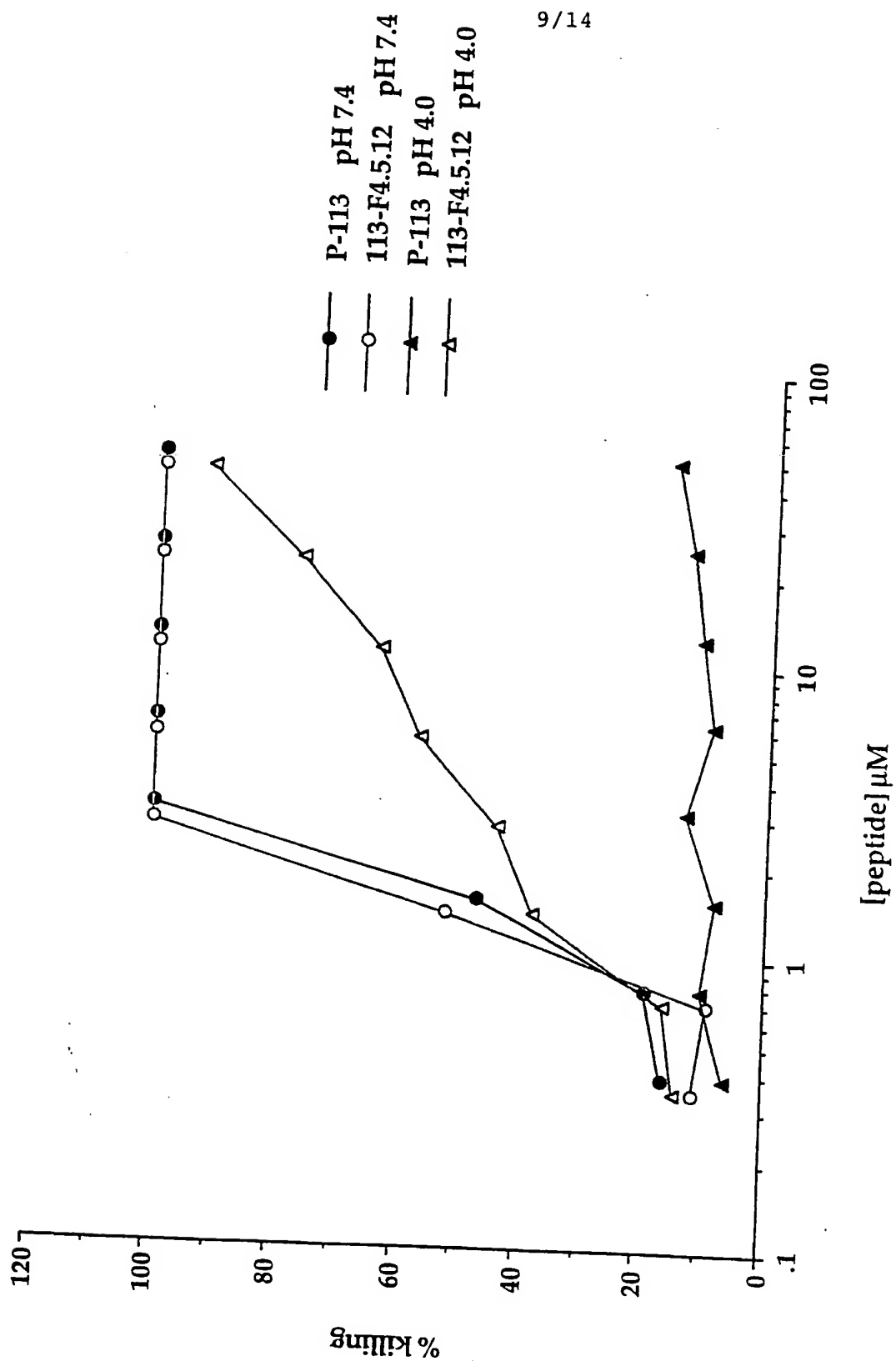


FIGURE 6

10/14

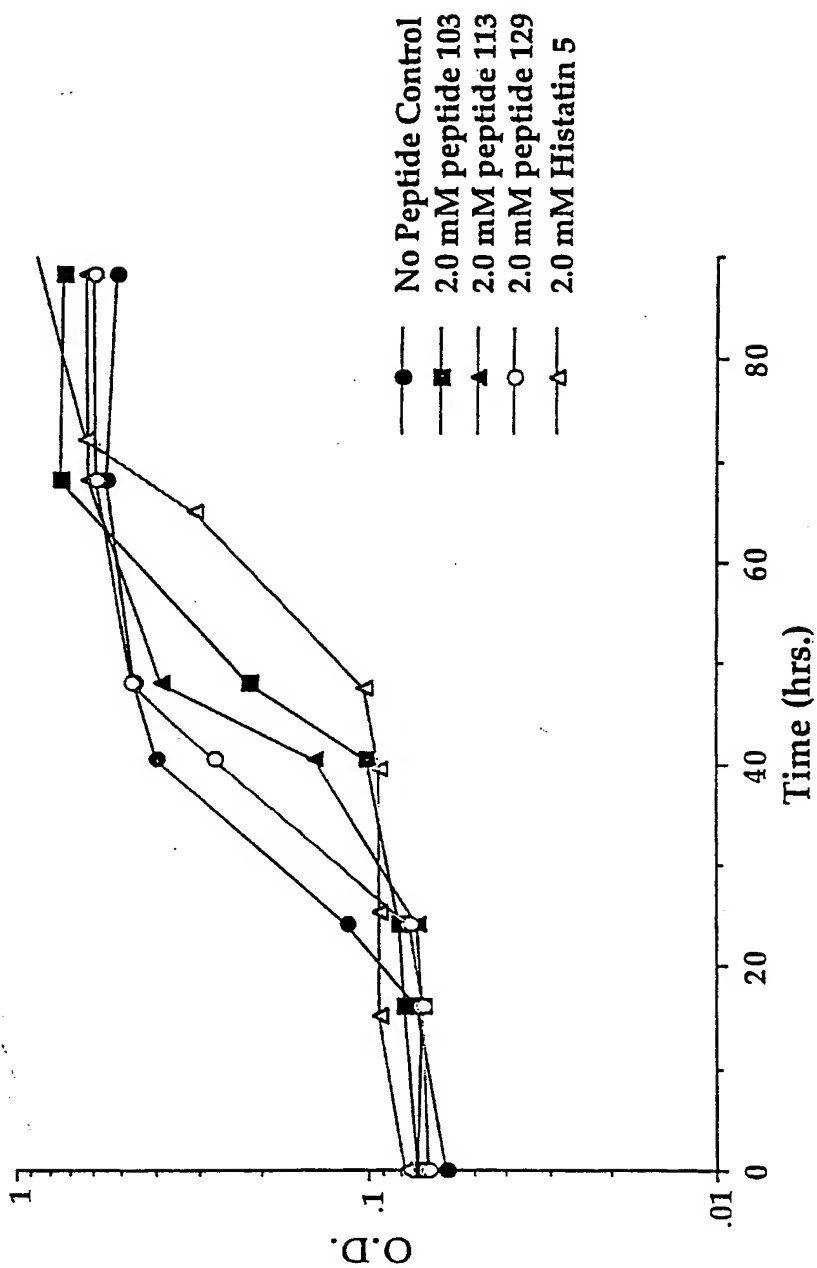


FIGURE 7

11/14

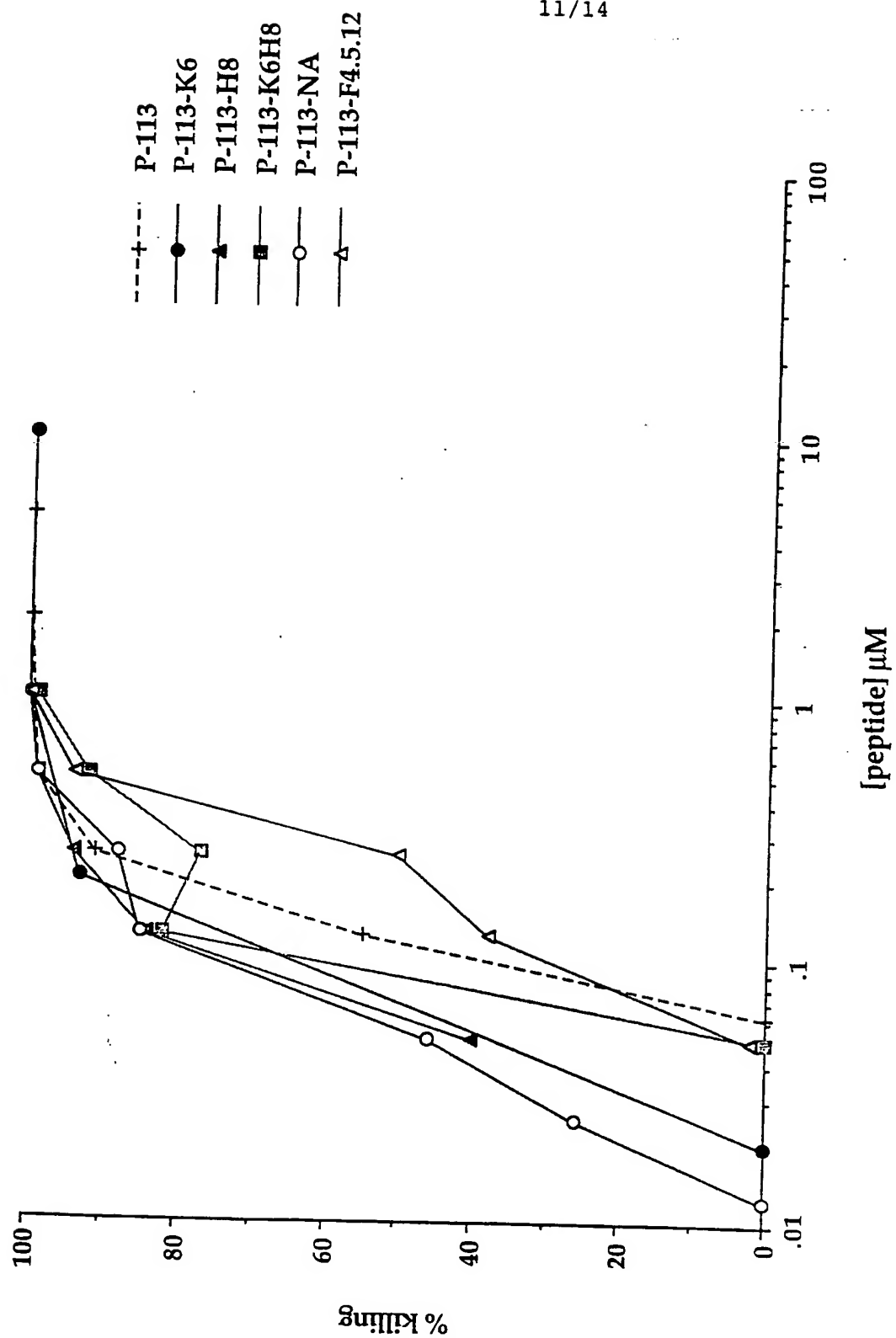


FIGURE 8

12/14

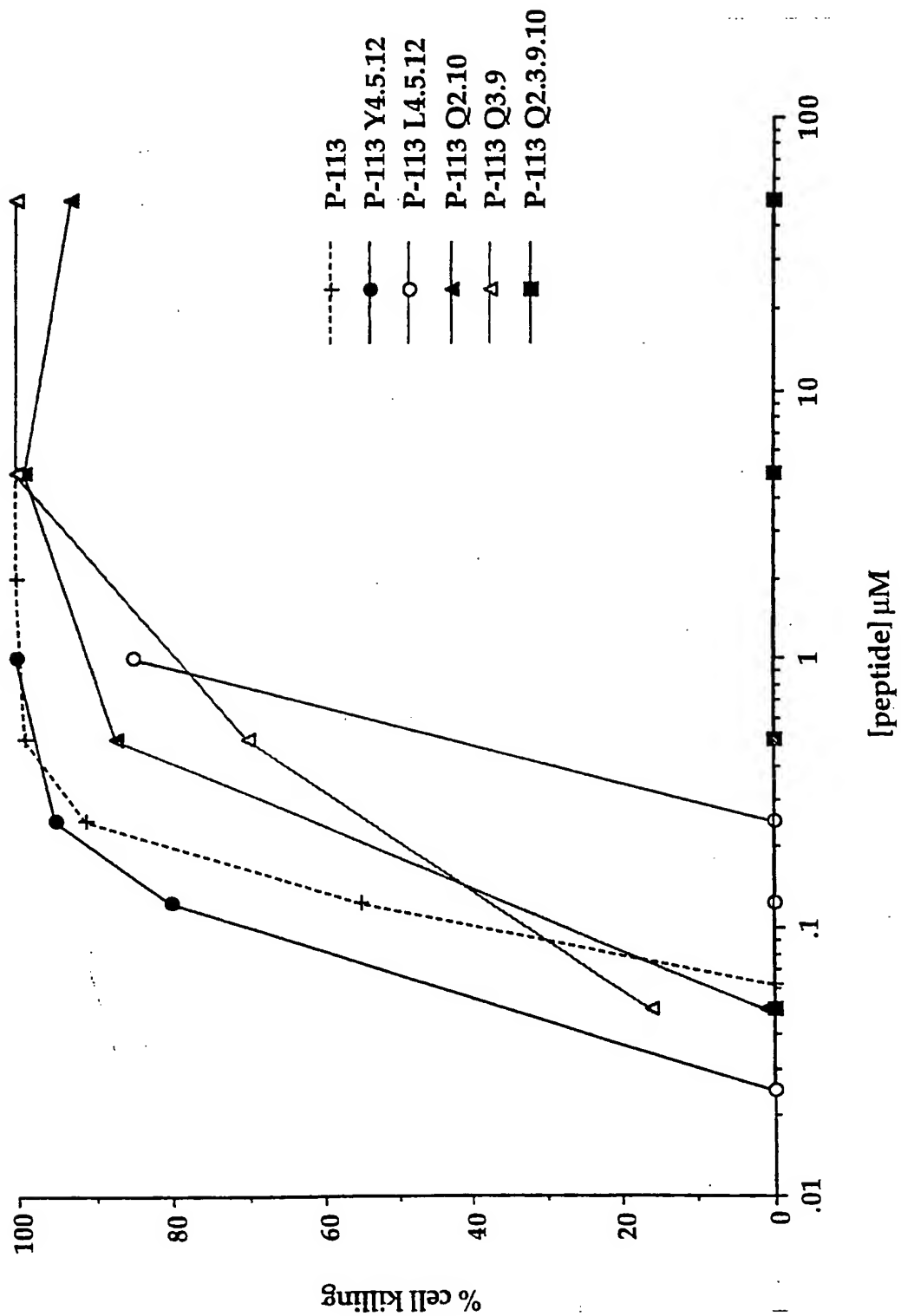


FIGURE 9

13/14

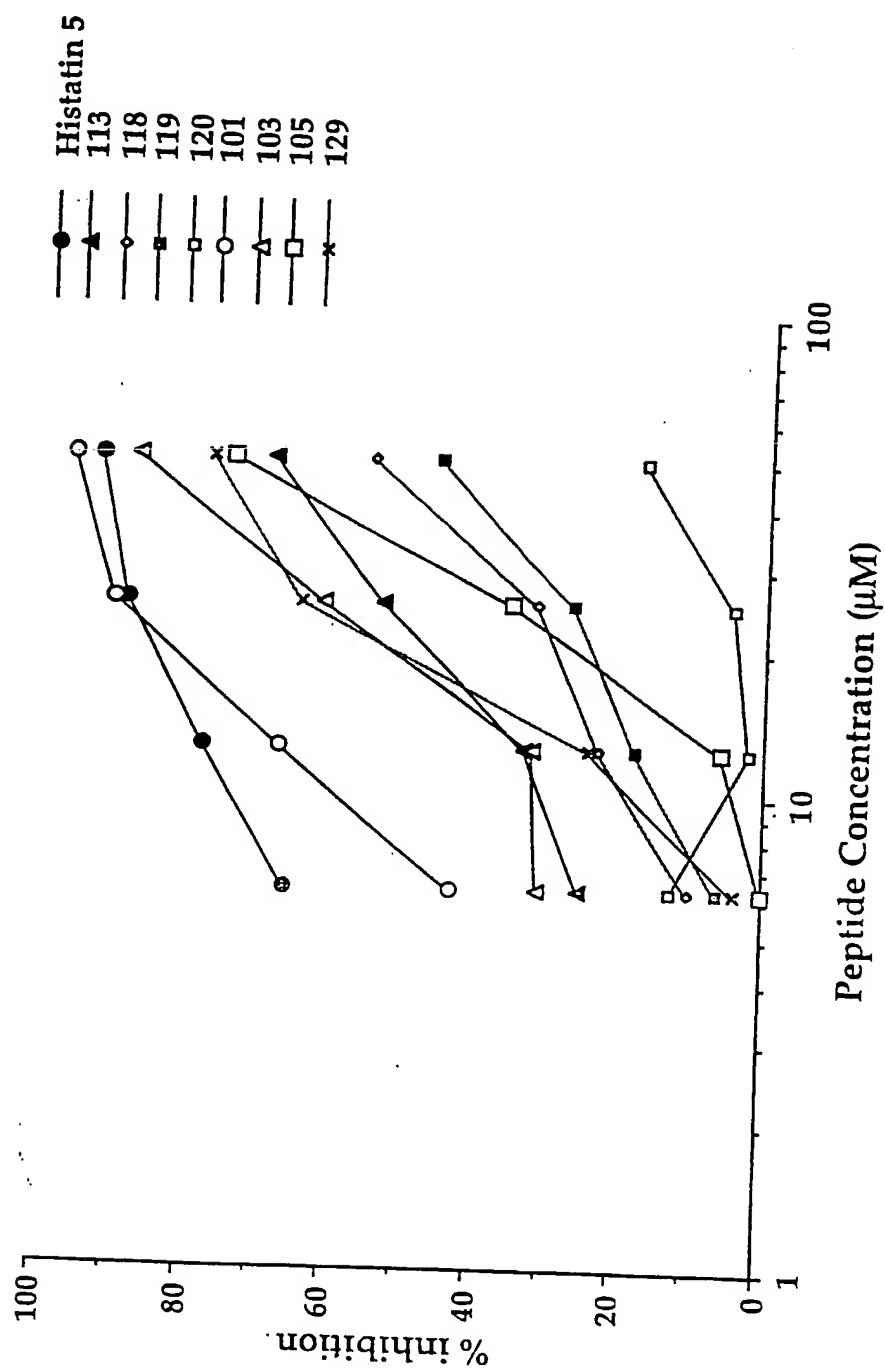


FIGURE 10

14/14

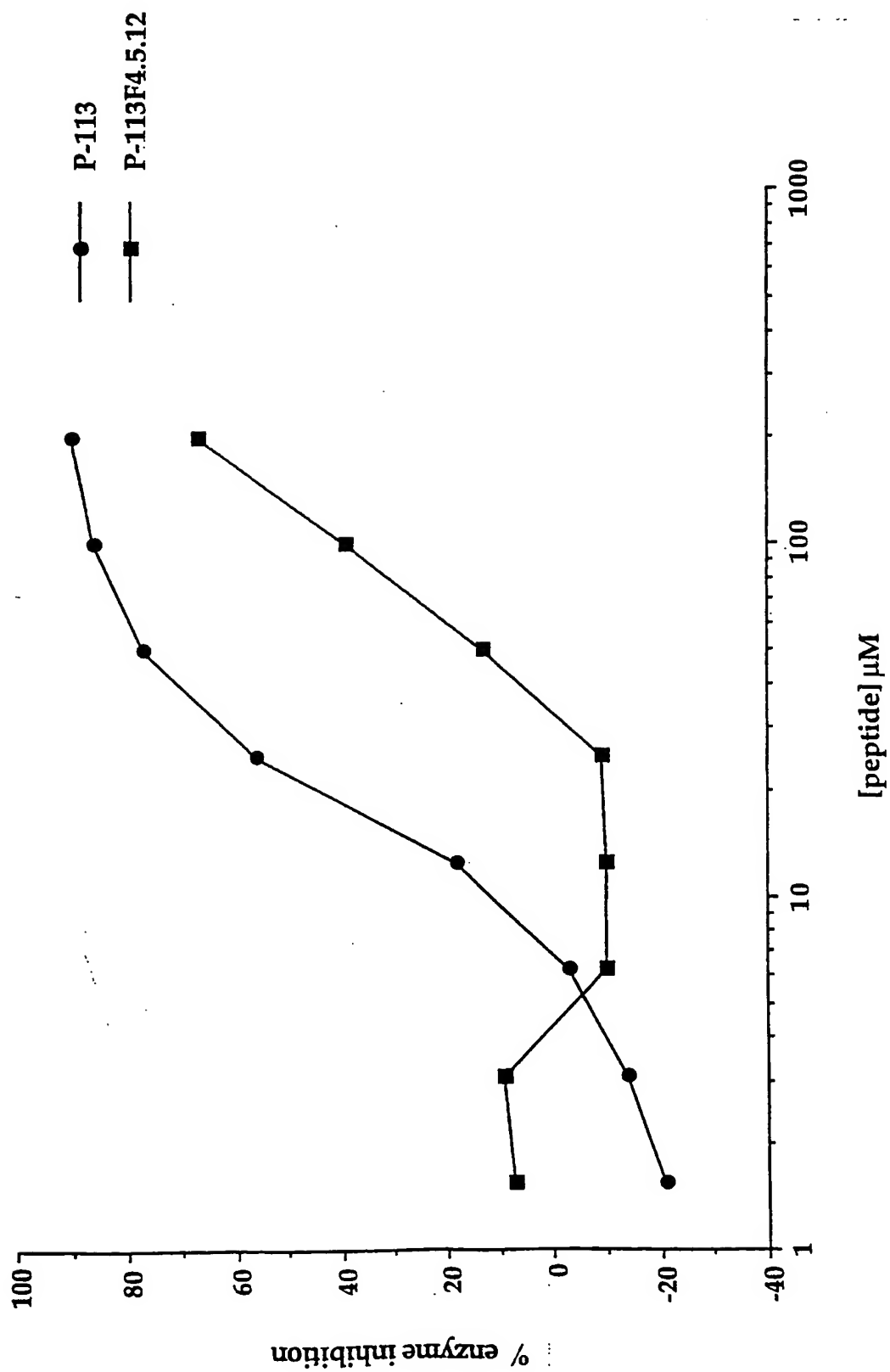


FIGURE 11

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/47, A61K 38/17		A3	(11) International Publication Number: WO 96/40768																																																																											
			(43) International Publication Date: 19 December 1996 (19.12.96)																																																																											
(21) International Application Number: PCT/US96/09374		FRIDEN, Phillip, M. [US/US]; 32 Washington Street, Bedford, MA 01730 (US).																																																																												
(22) International Filing Date: 7 June 1996 (07.06.96)		(74) Agents: BROOK, David, E. et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).																																																																												
(30) Priority Data: 08/481,888 7 June 1995 (07.06.95) US		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).																																																																												
(60) Parent Application or Grant (63) Related by Continuation US Not furnished (CIP) Filed on Not furnished		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>																																																																												
(71) Applicants (for all designated States except US): PERIODONTIX, INC. [US/US]; 313 Pleasant Street, Watertown, MA 02171 (US). TRUSTEES OF BOSTON UNIVERSITY [US/US]; 147 Bay State Road, Boston, MA 02115 (US).		(88) Date of publication of the international search report: 6 March 1997 (06.03.97)																																																																												
(72) Inventors; and																																																																														
(75) Inventors/Applicants (for US only): OPPENHEIM, Frank, G. [US/US]; 10 Heath Wood Lane, Chestnut Hill, MA 02167 (US). XU, Tao [CN/US]; 160 Pine Street, No. 9, Newton, MA 02166 (US). ROBERTS, F., Donald [US/US]; 2 Bridle Path Circle, Dover, MA 02030 (US). SPACCIAPOLI, Peter [US/US]; 9 High Road, Newbury, MA 01951 (US).																																																																														
(54) Title: ANTI-FUNGAL AND ANTI-BACTERIAL HISTATIN-BASED PEPTIDES																																																																														
<table><tr><td>Histatin 1:</td><td>1</td><td>5</td><td>10</td><td>15</td></tr><tr><td>Histatin 2:</td><td colspan="4">Asp-Phe-His-Glu-Lys-Arg-His-His-Gly-Tyr-Arg-Arg-Lys-Phe-His-Glu-Lys-His-His-Arg-Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Histatin 3:</td><td>1</td><td>5</td><td>10</td><td>15</td></tr><tr><td>Histatin 4:</td><td colspan="4">Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-Arg-Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Histatin 5:</td><td colspan="4">Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Histatin 6:</td><td colspan="4">Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Histatin 7:</td><td colspan="4">Arg-Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Histatin 8:</td><td colspan="4">Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Histatin 9:</td><td colspan="4">Arg-Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Histatin 10:</td><td colspan="4">Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Histatin 11:</td><td colspan="4">Lys-Arg-His-His-Gly-Tyr-Lys-Arg</td></tr><tr><td>Histatin 12:</td><td colspan="4">Lys-Arg-His-His-Gly-Tyr-Lys</td></tr><tr><td>Peptide 101:</td><td colspan="4">Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Peptide 102:</td><td colspan="4">Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td></tr><tr><td>Peptide 103:</td><td colspan="4">Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-</td></tr></table>				Histatin 1:	1	5	10	15	Histatin 2:	Asp-Phe-His-Glu-Lys-Arg-His-His-Gly-Tyr-Arg-Arg-Lys-Phe-His-Glu-Lys-His-His-Arg-Lys-Phe-His-Glu-Lys-His-His-				Histatin 3:	1	5	10	15	Histatin 4:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-Arg-Lys-Phe-His-Glu-Lys-His-His-				Histatin 5:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-				Histatin 6:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-				Histatin 7:	Arg-Lys-Phe-His-Glu-Lys-His-His-				Histatin 8:	Lys-Phe-His-Glu-Lys-His-His-				Histatin 9:	Arg-Lys-Phe-His-Glu-Lys-His-His-				Histatin 10:	Lys-Phe-His-Glu-Lys-His-His-				Histatin 11:	Lys-Arg-His-His-Gly-Tyr-Lys-Arg				Histatin 12:	Lys-Arg-His-His-Gly-Tyr-Lys				Peptide 101:	Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-				Peptide 102:	Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-				Peptide 103:	Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-			
Histatin 1:	1	5	10	15																																																																										
Histatin 2:	Asp-Phe-His-Glu-Lys-Arg-His-His-Gly-Tyr-Arg-Arg-Lys-Phe-His-Glu-Lys-His-His-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																													
Histatin 3:	1	5	10	15																																																																										
Histatin 4:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																													
Histatin 5:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																													
Histatin 6:	Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																													
Histatin 7:	Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																													
Histatin 8:	Lys-Phe-His-Glu-Lys-His-His-																																																																													
Histatin 9:	Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																													
Histatin 10:	Lys-Phe-His-Glu-Lys-His-His-																																																																													
Histatin 11:	Lys-Arg-His-His-Gly-Tyr-Lys-Arg																																																																													
Histatin 12:	Lys-Arg-His-His-Gly-Tyr-Lys																																																																													
Peptide 101:	Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																													
Peptide 102:	Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																													
Peptide 103:	Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-																																																																													
(57) Abstract																																																																														
Histatin-based peptides representing defined portions of the amino acid sequences of naturally occurring human histatins and methods for treatment of fungal or bacterial infection are described. These histatin-based peptides represent the active anti-fungal and anti-bacterial region of naturally occurring human histatins.																																																																														

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/US 96/09374

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/47 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94 21672 A (UNIV CALIFORNIA) 29 September 1994 see page 14, line 14 - line 19 ---	1-14
Y	JOURNAL OF DENTAL RESEARCH, vol. 69, no. 1, January 1990, pages 2-6, XP000611621 R.F. TROXLER ET AL.: "Structural relationship between human salivary histatins" see page 2, right-hand column, paragraph 1 see page 5, left-hand column, last paragraph - page 6, left-hand column, paragraph 1; figure 3 --- -/--	1-14



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

4 December 1996

Date of mailing of the international search report

31.01.97

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patendaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

FUHR, C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/09374

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 5 486 503 A (OPPENHEIM FRANK G ET AL) 23 January 1996 cited in the application see claims; examples ---	1-14
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 265, no. 7, 5 March 1990, BALTIMORE, MD US, pages 3898-3905, XP002020121 P.A. RAJ ET AL.: "Salivary Histatin 5: Dependence of Sequence, Chain Length, and Helical Conformation for Candidacidal Activity" see figures 1,6 -----	1-14

INTERNATIONAL SEARCH REPORT

ernational application No.

PCT/US 96/09374

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 9 - 14
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/09374

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9421672	29-09-94	US-A- 5459235	17-10-95
		AU-A- 6523994	11-10-94
		CA-A- 2155739	29-09-94
		EP-A- 0689550	03-01-96
		JP-T- 8508165	03-09-96

US-A-5486503	23-01-96	NONE	
